CHAPTER 3

AGE-RELATED FLIGHT AND REPRODUCTIVE PERFORMANCE

3.1 Introduction

Age-related changes in flight ability have been documented for a wide range of insect species (see reviews by Johnson 1969, Dingle 1985). A common feature of these studies has been the identification of a period of increased flight ability over a varying number of nights following emergence. The pattern of age-related flight performance varies among species; some exhibit a distinct peak while in others maximum flight may plateau for severals days after an initial rise.

Many insect species similarly display age-related changes in reproductive performance (Engelmann 1970, Boggs 1986). The rate of reproductive development may vary, eggs may be mature at the time of adult eclosion or there may be a maturation phase occurring over a number of days following emergence. Temporal patterning of reproductive effort may also occur, suggesting age specific fecundities.

How the two systems are interrelated is largely a function of the life-history pattern of the species concerned. Among noctuids, including *Helicoverpa* spp., there is substantial evidence indicating that migration is undertaken by post-teneral prereproductives (Gatehouse 1989, Farrow & Daly 1987, Fitt 1989), though there is also some evidence for considerable movement by reproductively mature moths (Haggis 1981). Studies of age mediated changes in flight capacity of *H. armigera* from the Sudan (Hackett & Gatehouse 1982) and from India (Armes & Cooter 1991) show a general increase in flight ability during the first 4 to 6 nights following emergence, coinciding with the onset of egg laying.

Other factors, such as mating and availability of adult food sources, may also potentially influence the expression of age-related flight and reproductive capacity. Generally mating has been shown to inhibit subsequent flight activity (Dingle 1985), though mating may also promote flight activity as has been shown for the pyralid,

Chilo partellus (Pats & Wiktelius, 1989). For *H. armigera,* tethered flight experiments have shown either no influence of mating on flight (Hackett & Gatehouse 1982) or suppression of flight capacity (Armes & Cooter 1991). Mating, however, has been widely shown to enhance maturation and deposition of eggs among heliothine moths, including *H. punctigera* and *H. armigera* (reviewed by Zalucki *et al.* 1986).

This chapter aims to examine age-related changes in flight and reproductive capacity of *Helicoverpa punctigera* and *H. armigera,* and to assess the impact of mating and adult food availability on these parameters.

3.2 Materials and methods

3.2.1 Larval and adult rearing

Laboratory cultures of *H. punctigera* and *H. armigera* were established from field collected larvae from the Armidale region of the New England Tablelands, New South Wales, Australia. Larvae of *H. punctigera* were collected during November 1987, and those of *H. armigera* during January 1988. Insect material was bred for one generation in the laboratory prior to experimentation. Larvae of both species were reared individually in 35 ml plastic cups at a constant temperature of 24 ± 1° C under a light regime of 14L: 10D. Relative humidity was maintained at 60 - 70%. Larvae were reared on an artificial diet based on the formula provided by Teakle & Jensen (1985).

Pupation occurred within the larval rearing container amongst excess diet and frass. Following pupation, pupae were removed, sexed and stored in groups of 10 - 12 individuals in clear 125 ml plastic cups. Upon emergence, adults were transferred to, and maintained individually within, 285 ml plastic cups. Paper towelling was provided as a substrate for moths to cling to, and as an oviposition surface for females. A 10 % honey solution was supplied via soaked cotton wool plugs fitted to glass vials and renewed daily. Pupae and adults were maintained under the same conditions as those for rearing larvae.

3.2.2 Tethered flight methodology

Moths were flight tested using a tethered technique based on that of Dingle (1965). In this method adults were suspended from a hinged vertical rod so that loss of tarsal contact would initiate flight. The hinge enabled forward motion and a degree of lateral movement of the tethering arm. Moths were anaesthetized once using diethyl ether to allow attachment of the tether, which consisted of a 5mm length of polyethylene tubing mounted dorsally on the thorax with a contact adhesive. The polyethylene tubing provided a friction fit to the vertical tethering arm. The tether remained in position throughout the insects' life. Moths were tethered to the vertical rod only during periods of flight testing.

Flight tests were initiated within 45 minutes of the commencement of the dark phase (sunset), as this period coincides with the onset of flight activity of both species in the field (Drake & Farrow 1985). Flight duration was measured from the time that moths were started flying to when moths voluntarily stopped flying. A moth was deemed to have stopped flying after an arbitarily defined period of 15 sec immobility. Moths were then stimulated by gentle tapping of the tethering arm to initiate further flight.

3.2.3 Determining relationship between age and flight ability

Individual moths were flight tested on successive nights during the first 12 nights post emergence. Davis (1980) demonstrated the need to test individuals on successive nights in order to identify the presence of long fliers. Restricting flight testing to one night only may significantly underestimate the proportion of long fliers. On each night individual moths were either flight tested over a period of 5 hours, or the duration of the first 5 consecutive flights was recorded, whichever occurred sooner. Flights in excess of 5 hours were stopped and moths returned to holding containers. A period of 5 hr continuous flight was thought sufficient to assay relative changes in flight duration with age. Previous tethered flight studies of noctuids have defined long

(migratory) flights as those moths with a total flight duration of more than 2 hours (Hackett & Gatehouse 1982, Parker & Gatehouse 1985). Flight testing was carried out under dim light in a room at a temperature of 24 - 26 °C. Data used to construct age-related flight profiles were from unmated males and females. Holding containers were provided with honey solution and paper towelling as an oviposition surface. The honey solution and paper towelling was renewed daily. Numbers of eggs laid by each moth were counted on a daily basis. Time to first oviposition, fecundity and longevity were recorded.

Survival analyses (Dixon 1981) were used to describe the distribution of flight durations for each test night. The analysis accommodated censored data, that is, flights which were terminated at 5 hr duration. The equality of flight distributions were tested (male - female comparisons) using the generalised Wilcoxon (Breslow) statistic. Median flight durations (± s.e) were used to construct age-related flight duration profiles.

3.2.4 Mating and flight ability

To determine the effect of mated status on flight duration of females, newly emerged *H. punctigera* and *H. armigera* were maintained as single male and female pairs or as individual females. These were held under the same environmental conditions as detailed in section 3.2.1. Moths were supplied with 10 % honey solution from the time of emergence which was renewed daily. Females were then flown on nights 4, 6, 8 or 10 post emergence. Females were flown over a 3 hr test period and categorised in flight durations of < 60, 61 - 120, 121 - 180 and > 180 min. Those paired with males were dissected after flight testing to determine their mated status, by counting the number of spermatophores. Age at mating was not determined for these moths, though presumably occurring only once females commenced calling. The influence of mating on flight duration of males was not investigated for either species.

3.2.5 Adult food and flight ability

The influence of adult food availability on tethered-flight duration was determined by comparing flight durations of honey and water fed females. Moths were supplied with either 10 % honey solution or distilled water from the time of emergence. This was supplied in the same manner as described above (section 3.2.1). Moths were flown over a 3 hr test period on nights 1, 2, 4, 6 or 8 and classified under the same flight duration categories as used in section 3.2.4.

3.2.6 The influence of age and adult food availability on reproductive performance

The influence of mating on fecundity and oviposition rate was examined by comparing daily egg laying of 25 mated and 25 unmated honey fed *H.armigera* females, 25 unmated water fed *H.armigera* females, and 29 mated and 29 unmated honey fed *H. punctigera* females and 29 unmated water fed *H. punctigera* females. Time to first oviposition, daily egg counts and lifespan were recorded. Those females assigned to a treatment involving mating were paired on the morning following emergence, and until death, with one male of age 2 - 4 days. *Helicoverpa* males are reproductively mature at the age of 2 days (Zalucki *et. al.* 1986). Dependent on the treatment group moths were fed either 10 % honey solution or water from the time of emergence; this was renewed daily. Moths were held in clear 285 ml plastic containers, and supplied with paper towelling as an oviposition surface. Each day, the paper towelling was removed, the number of eggs laid overnight counted and a fresh piece of towelling provided. This was continued until each female moth died. Successful mating was indicated by the deposition of fertile eggs; these eggs were retained and observed for up to 4 days and showed recognisable signs of embryonic development as egg color changed from white, through brown to black (Zalucki *et. al.* 1986). Failure to mate was indicated by the converse of the above; eggs appeared normal when newly laid; however, within 2 - 3 days they yellowed and collapsed. Those moths which

were paired with a male but failed to successfully mate were not included in the analysis.

3.3 Results

3.3.1 Flight and age

Preliminary analysis of tethered flight data indicated that on each night both *H. armigera* and *H. punctigera* moths undertook one long duration flight. Subsequent flights were of shorter duration. The longest flight was typically the first flight following the release of tarsal contact. Figs 3.1a and b show median flight durations for *H. armigera* males and females on nights 1, 2, 4, 6, and 8 that completed between 2 to 5 flights within the 5 hour test period on each of these nights. Figs 3.2a and b show similar data for *H. punctigera* males and females. For each moth, flights were ranked from the longest (designated flight 1) to the shortest (designated flight 2, 3, 4 or 5). Median flight durations (mins) were computed for each flight. Analysis of variance by ranks (Kruskal-Wallis test) indicated that the longest flight was significantly greater than the duration of subsequent flights for both *H. armigera* ($P < 0.001$, pooled data for males and females and for nights 1, 2, 4, 6, and 8) and *H. punctigera* ($P < 0.001$, for data as above). The duration of the longest flight was subsequently used to construct median flight profiles and flight distribution profiles for each species.

Median flight durations (± s.e) for *H. armigera* males (n = 30) and females (n = 30), and *H. punctigera* males (n = 38) and females (n = 35) are shown in Figs 3.3a and b. For *H. armigera,* flight duration of both sexes increased in early adult life to peak on night 4. Flight durations declined after this period in both sexes. For *H. punctigera,* median flight durations of females increased gradually from night 1 to peak on night 4. Flight durations remained elevated but variable for the remaining 8 nights. For *H. punctigera* males, there was no clearly defined peak in flight duration. Median flight durations increased gradually to be highest on night 10.

Fig 3.4 shows the proportions of *H. armigera* males and females flying, versus flight duration (min) on nights 1, 2, 3, 4, 6 and 8. On night 1 the majority of flights

were of less than 60 min duration (males - 79 %, females - 86 %). No males, and only 3 % of females completed flights exceeding 5 hr duration on this night. Between nights 2 and 4 the proportions of both males and females undertaking single flights exceeding 5 hr duration progressively increased. On night 4, 39 % of females and 46 % of males completed a single flight exceeding 5 hr duration. By nights 6 and 8 flights shifted to shorter duration, with < 10 % of either sex flying for more than 5 hr. Statistical comparisons were made between male and female flight distributions on each of these nights. No significant difference was indicated on night 1 (Breslow = 3.06, $P > 0.05$), but significant differences occurred on night 2 (Breslow = 10.56, $P < 0.01$), night 3 (Breslow = 5.99, $P < 0.05$), night 4 (Breslow = 4.89, $P < 0.05$), night 6 (Breslow = 12.6, $P < 0.05$) and night 8 (Breslow = 4.7, $P < 0.05$). On each of these nights a greater proportion of males undertook long flights (> 3 hr) than females.

Proportions of *H. punctigera* males and females flying versus flight duration (min) on nights 1, 2, 3, 4, 6, 8 and 10 are shown in Fig 3.5. For both males and females, the majority of flights (approx. 80 %) did not exceed 60 min duration on any one night. A small proportion (< 10 %) of both males and females flew in excess of 5 hr duration on these nights. On night 8, approximately 20 % of males flew for between 2 and 3 hr, with no moths flying for more than 4 hr. Comparison of male and female flight distributions on these nights indicated no significant differences on any night ($P > 0.1$ in each case).

3.3.2 Pre-oviposition period, fecundity and longevity of flown moths

There was no indication of a relationship between flight duration and pre-oviposition period for either *H. armigera* or *H. punctigera* females. For *H. armigera* 33.3 % (n = 10) and 76.7 % (n = 23) of unmated females were ovipositing by nights 2 and 3. For *H. punctigera* 82.9 % (n = 29) and 91.4 % (n = 31) of unmated females were ovipositing by nights 2 and 3 (Table 3.1). The pre-oviposition periods, fecundities and longevities of these moths are presented in Table 3.2. Time to first oviposition in *H. armigera* females was significantly greater ($t_{63} = 3.98$, $P < 0.001$)

than preoviposition period in *H. punctigera*. Significant differences were not evident between fecundities ($t_{63} = 0.15$, $P > 0.8$) or longevities ($t_{63} = 0.49$, $P > 0.5$) of unmated flight tested *H. armigera* and *H. punctigera* females.

3.3.3 Mating and flight duration

Tables 3.3 and 3.4 give the percentages of mated and unmated *H. punctigera* and *H. armigera* females in each flight category flown on nights 4, 6, 8, or 10. For *H. punctigera,* though there was a consistent trend for fewer mated moths to complete flights of more than 3 hours on each night, chi-square analyses did not indicate significant differences on nights 4, 6 or 8. On night 10, however, a significant difference ($P < 0.05$) was detected between numbers of mated and unmated moths among these flight categories. Subdivision of the contingency table (Zar 1974) indicated a significant reduction in the number of mated moths on night 10 undertaking flights longer than 3 hours as compared with unmated moths ($P < 0.02$).

For *H. armigera,* chi-square analyses indicated no significant differences in the numbers of moths in each flight category on nights 4, 6 or 8. On night 10, however, a significant difference ($P < 0.02$) was detected between numbers of mated and unmated moths within these flight categories. Subdivision of the contingency table indicated a significant ($P < 0.02$) decrease in the number of mated moths completing flights longer than 3 hours, when compared with unmated moths.

3.3.4 Adult food availability and flight duration

Tables 3.5 and 3.6 give the percentages of honey and water fed *H. punctigera* and *H. armigera* females in each flight category flown on nights 1, 2, 4, 6 and 8. For *H. punctigera,* chi-square analyses did not identify significant differences between flight durations of honey and water fed moths on nights 1, 2, 4 or 6. On night 8, however, significant differences were detected. Subdivision of the contingency table (Zar 1974) showed that significantly fewer ($P < 0.05$) water fed moths on night 8 were exhibiting flights of greater than 180 min duration as compared with honey fed moths.

For *H. armigera,* no significant differences were detected in the numbers of moths in each flight category on nights 1 or 2. On nights 4, 6 and 8, however, significant differences were detected between numbers of water and honey fed moths in these flight duration categories. On each of these nights, there were significantly ($P < 0.05$ in each case) fewer flights exceeding 3 hr duration by water fed than honey fed moths. On night 6 no water fed females flew for more than 3 hr and on night 8 none flew for more than 2 hr.

3.3.5 Age-related reproductive performance

Oviposition profiles for mated and unmated *H. armigera* moths fed with honey are shown in Fig 3.6a, and oviposition profiles for mated and unmated *H. punctigera* moths fed with honey are shown in Fig 3.6b . In both species mating clearly increased the fecundity and the rate of egg laying during early adult life. For mated *H. punctigera* females oviposition peaked on nights 4 - 5, whereas for *H. armigera* this was on nights 5 - 8. Data on the effects of mating and feeding on preoviposition period, lifetime fecundity and longevity are presented in Table 3.7. Single factor analysis of variance (data grouped for interspecific comparison) (Zar 1974) indicated significant differences in fecundities ($F_{4,132} = 19.4$, $P < 0.001$) and longevities ($F_{4,132} = 53.9$, $P < 0.001$), but not pre-oviposition periods ($F_{4,132} = 0.85$, $P > 0.1$) between treatment groups. Mating had a significant (S.N.K test, $P < 0.05$) positive effect on the fecundity of *H. punctigera* and *H. armigera.* Fecundities of mated females were significantly greater than that of unmated females (see Table 3.7). There was a significant (S.N.K. test, $P < 0.05$) negative effect of mating on longevity of *H. punctigera* and *H. armigera* females.

Restricting adult food supply did not influence the onset of oviposition in *H. punctigera* females, whereas in *H. armigera* females, moths fed with water commenced oviposition significantly later (mean of 4.6 nights after emergence) than moths fed with honey (mean of 1.9 nights after emergence) ($P < 0.05$). Fecundities of moths fed with water were significantly ($P < 0.05$) less than those of moths fed with

honey for both species. Provision of honey significantly ($P < 0.05$) increased longevity in both species (see Table 3.7).

3.4 Discussion

In common with other studies of insect tethered-flight (Davis 1980, Dingle 1985, Armes & Cooter 1991, Sappington & Showers 1991) there is great variation in the duration of flights between individuals and between nights. Also in common with previous studies, is the skew in the distribution of flight durations towards short flights. Many individuals fly for short periods and only a few make long flights (see Figs 3.4 and 3.5)

The results of this study show that the tethered-flight durations of both species increase during early adult life. Peak median flight durations occurred on night 4 for *H. armigera* males and females, but declined rapidly after that night. For *H. punctigera* females median flight duration also peaked on night 4, but remained elevated for the remainder of the test period. For *H. punctigera* males median flight durations increased gradually throughout the 12 nights on which moths were flight tested. Long flying moths (> 5 hr duration) were evident in both species from night 1 and in varying proportions throughout the following 12 nights on which moths were flight tested. Other tethered-flight studies of noctuids, including those on *Helicoverpa* spp, have identified similar age-related changes in tethered-flight duration. Hackett & Gatehouse (1982) demonstrated a peak in flight ability on night 4 for *H. armigera* from the Sudan, and Armes & Cooter (1991) similarly identified a peak on night 4 for *H.armigera*, for a population from India. Further, Sappington & Showers (1991) identified a peak in tethered flight ability on night 3 for the noctuid *Agrotis ipsilon*.

How age-related changes in flight ability of *H. punctigera* and *H.armigera* moths are expressed under free flight conditions in the field have not been determined. It has generally been assumed in other tethered-flight studies that the age at which maximum flight capacity occurs corresponds to the migratory period for the particular test species (Johnson 1969). Data from tower light trapping, however,

suggests that long distance migratory flights by *H. punctigera* and *H. armigera* are undertaken by reproductively immature moths (see Ch. 2), though it is not known for how many nights moths migrate as the oocytes mature. Given the rapid attainment of reproductive maturity recorded for both species in this study; most *H. punctigera* (91.5 %) and *H. armigera* (77.7 %) females that were flight tested commenced oviposition within 2 - 3 nights of emergence (see Table 3.1); migratory flights would have to occur at least prior to night 3. It is likely, therefore, that the long flying moths present on nights 1 and 2 following emergence are those individuals with the potential to undertake long distance migratory flights. Armes & Cooter (1991) similarly demonstrated the rapid attainment of reproductive maturity by *H. armigera* females with 83 % of moths ovipositing by night 3. Observations of emigrating *Helicoverpa* adults from emergence sites containing both nectar sources and potential larval food plants suggested that as few as 10 % of moths undertake migratory flights (Schaefer 1976, cited in Fitt 1989). During this study comparably low numbers of moths (3 - 18 % of *H. armigera* and 4 % of *H. punctigera*) displayed long duration flights (> 5 hr duration) during nights 1 - 2. The tethered-flight profiles generated for *H. punctigera* and *H. armigera* from this study, given the generally short pre-oviposition periods for both species, are predominately those of reproductively mature moths. The observed increase in the flight ability of *H. punctigera* and *H. armigera* adults to nights 4 - 5, therefore, seems to be associated with movement within the flight boundary layer for vegetative (*sensu* Kennedy 1975) purposes rather than for migration.

Though tethered-flight studies have been used as assays for the identification of migratory behaviour (Dingle 1985, Rankin *et. al.* 1986), the technique employed here, though similar in many aspects to other tethered flight methodologies (reviewed by Dingle 1986) does not specifically incorporate a behavioural component, that is, moths are forced to fly rather than being allowed to initiate flight by their own volition. The technique employed in this study measures locomotor performance (flight duration) and as such allows a relative estimate of experimental treatments on this parameter. The

technique hence does not allow the differentiation between appetitive versus migratory flights (Kennedy 1975).

For *H. punctigera* and *H. armigera* significant differences in flight ability between mated and unmated moths were not detected until night 10. Flight ability of mated moths on this night was significantly reduced, as indicated by the number of moths completing flights exceeding 3 hours duration, in comparison with unmated moths. In this study, mating clearly increased the oviposition rate of females at the expense of longevity. The decline in flight ability between mated and unmated moths, therefore, appears to be a consequence of the decline in energy reserves caused by the increased maturation and deposition of eggs that follows mating. In *H. punctigera,* though there was no significant differences in flight duration of mated and unmated females during early adult life, there was a consistent trend for fewer mated moths to undertake flights exceeding 3 hours duration on the nights 4, 6 and 8. There was, however, no comparable trend for *H. armigera* females. Hackett & Gatehouse (1982) were also unable to detect any effect of mating on flight duration of *H. armigera* females during early adult life. The trend for decreased flight ability by mated *H. punctigera* females is consistent with the findings on *H. armigera* by Armes & Cooter (1991).

Denying adults access to a carbohydrate source did not significantly influence tethered-flight duration on nights 1 or 2 for either *H. punctigera* or *H. armigera* females. For *H. punctigera* moths flown on nights 4 and 6, there was a consistent trend for greater numbers of *H. punctigera* moths fed with honey to undertake flights of more than 3 hours duration in comparison with moths fed with water. The differences, however, were not significant. Significant differences in flight duration, however, occurred on night 8 for *H. punctigera* and nights 4, 6 and 8 for *H. armigera.* On each of these nights the numbers of honey fed moths flying for more than 3 hours duration exceeded those of water fed moths flying for the same duration. As both species continue to produce eggs when denied access to a carbohydrate source, these differences may be a consequence of the depletion of energy reserves required to

maintain both reproductive output and flight capability (Willers *et al.* 1987). Restricting the availability of carbohydrate during early adult life (nights 1 - 2 following emergence) has previously been shown to increase the incidence of long duration (migratory) flights (Hackett & Gatehouse 1982), coinciding with an apparent delay in reproductive maturity. There was no indication during this study in either *H. punctigera* or *H. armigera* , that moths denied access to carbohydrate during this stage exhibited longer duration flights than moths provided with carbohydrate, though *H. armigera,* but not *H. punctigera* females, exhibited a delay in the onset of reproductive activity (as marked by the deposition of eggs). The results of this study indicate that any observed effect on flight duration resulting from restricting carbohydrate availability is likely to be a consequence of the depletion of energy reserves (Willers *et. al.* 1987).

Few differences are evident when comparing the reproductive capacities of *H. punctigera* and *H. armigera* females recorded in this study. The mean fecundity of mated *H. punctigera* (1619 eggs per female) though greater than that of *H.armigera* females (1440 eggs per female) was not significantly different. There were similarly no significant differences between the fecundity of unmated *H. punctigera* (1120 eggs per female) and *H. armigera* (962 eggs per female) when provided with honey solution. The mean fecundity of *H. armigera* females fed with water (342 eggs per female), however, was significantly lower than the mean for *H. punctigera* females fed with water (703 eggs per female). Cullen (1969, cited in Zalucki *et. al.* 1986) has provided the only other account of reproductive performance in *H. punctigera.* Cullen recorded a mean fecundity of 1437 ± 229 eggs per female for mated *H. punctigera* held at 24° C. This is similar to the fecundity of *H. punctigera* females recorded in this study. No equivalent studies have been published for *H. armigera.*

Mating clearly increased the lifetime fecundities and the daily egg laying rates of both species, while reducing their longevities. The positive influence of mating on fecundity, at the expense of longevity, in *Helicoverpa* spp has been documented previously (Reed 1965, Proshold *et. al.* 1982). The deposition of eggs by unmated

females is also well documented (Proshold *et. al.* 1982, Zalucki *et. al.* 1986). In both species, though virgin females produce fewer eggs and at a slower rate than mated females (Fig. 3.4), their longevity is significantly greater and lifetime fecundity, while significantly lower than those of mated moths, nevertheless may be substantial, ranging from 138 to 1837 eggs per female for *H. armigera* and 232 to 2084 eggs per female for *H. punctigera*. This contrasts with the results of Lukefahr and Martin (1964) who found a five-fold decrease in the fecundities of water fed *H. virescens* and *Heliothis zea* moths in comparison with honey fed moths. Such apparent differences may represent interspecific differences in reproductive patterns within the heliothine moths as a group, or they may simply reflect differences in the way adults are maintained in the laboratory.

The results of this study indicate an apparent dichotomy in the pattern of oviposition between mated and unmated *Helicoverpa* females. In the former, the rate of egg deposition is greatly increased during early adult life, apparently at the expense of their longevities; whereas in unmated females, a moderate quantity of eggs are deposited and their longevities are increased (see Fig 3.6 and Table 3.7). The potential lifetime fecundity of *Helicoverpa* spp. (and other noctuids) is apparently fixed at emergence, whereby all the eggs that will be produced are present but immature (Proshold *et. al.* 1982). As a consequence of this, there may be some adaptive value for unmated moths in maintaining a relatively longer life expectancy while suppressing the maturation of a relatively large proportion of their available eggs. This may indirectly improve the probability of locating a mate (their flight ability is maintained) and at the same time maximise the number of eggs available for fertilisation.

Restricting adult food intake to water only further decreased the lifetime fecundities and longevities of females of both species (see Table 3.7). The deposition of eggs, though delayed in *H. armigera* females, did not differ significantly for *H. punctigera* females in comparison with moths fed with honey.

High fecundity combined with a short generation time has been identified as a major factor contributing to the pest status of *H. armigera* and *H. punctigera* (Fitt

1989). There are, however, no estimates of realised fecundity by *Helicoverpa* spp. in the field (Fitt 1989). How fecundities displayed under laboratory conditions relate to oviposition rates in the field have not been determined. Under natural field conditions egg placement by females is more selective (eg. Coombs & Ramsey 1991); they may therefore oviposit fewer eggs per night than do laboratory females. In addition females must expend more energy in searching for feeding and oviposition sites than do females held in laboratory cages. Realised fecundity in the field may also be influenced by larval host plant quality and the quality and quantity of nectar sources for the adult (Boggs 1986).

3.5 References

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Table 3.1 Percentages of unmated *H. punctigera* and *H. armigera* females ovipositing for the first time (as nights after emergence).

	Night				
	1	2	3	4	5
% *H. punctigera* n = 35	8.6	74.3	8.6	2.8	5.7
% *H. armigera* n = 30	3.3	30.0	43.4	20.0	3.3

Table 3.2 Pre-oviposition period, fecundity and longevity of unmated *H. punctigera* and *H. armigera* females flown on consecutive nights throughout their lifetime. Values represent mean ± s.d. (range).

	n	Preoviposition period (days)	Fecundity no. eggs / female	Longevity (days)
H. punctigera	36	1.2 ± 0.9^{a} (1 - 4)	746 ± 447^{a} (118 - 1743)	16.1 ± 4.0^{a} (9 - 25)
H. armigera	30	1.9 ± 0.7^{b} (1 - 3)	763 ± 414^{a} (88 - 1358)	14.6 ± 2.2^{a} (10 - 20)

Values followed by the differing superscripts differ significantly at $P < 0.05$ (t-test).

(within column comparisons only).

Table 3.3 The effect of mating on flight duration (min) of *H. punctigera* females on nights 4, 6, 8 and 10.

		Percentage of moths flying				
	n	< 60	61 - 120	121 - 180	> 180	Chi-sq
Night 4						
Mated	45	71.1	24.4	2.3	2.2	n.s.
Unmated	49	77.5	10.2	8.2	4.1	
Night 6						
Mated	57	57.9	15.8	1.7	24.6	n.s.
Unmated	56	39.3	19.6	7.2	33.9	
Night 8						
Mated	46	73.9	19.5	4.4	2.2	n.s.
Unmated	38	63.1	23.7	0	13.2	
Night 10						
Mated	56	89.3	5.3	3.6	1.8	$P < 0.05$
Unmated	40	65.0	12.5	10.0	12.5	

Table 3.4 The effect of mating on flight duration (min) of *H. armigera* females on nights 4, 6, 8 and 10.

		Percentage of moths flying				
	n	< 60	61 - 120	121 - 180	> 180	Chi-sq.
Night 4						
Mated	29	13.8	17.2	13.8	55.2	n.s.
Unmated	28	28.6	3.6	0	67.8	
Night 6						
Mated	26	34.6	19.2	11.6	34.6	n.s.
Unmated	26	53.7	11.5	4.1	30.7	
Night 8						
Mated	24	54.2	29.1	0	16.7	n.s.
Unmated	25	60.0	16.0	16.0	8.0	
Night 10						
Mated	26	73.0	15.4	7.8	3.8	$P < 0.02$
Unmated	27	62.9	3.7	11.2	22.2	

Table 3.5 The effect of adult food availability on flight duration (min) of *H. punctigera* females on nights 1, 2, 4, 6 and 8.

		Percentage of moths flying				
	n	< 60	61 - 120	121 -180	> 181	Chi-sq
Night 1						
Water	50	84.0	6.0	2.0	8.0	
Honey	45	66.6	20.0	6.7	6.7	n.s
Night 2						
Water	60	68.3	6.7	8.3	16.7	
Honey	65	72.3	9.2	6.2	12.3	n.s
Night 4						
Water	35	14.3	20.0	14.3	51.4	
Honey	42	7.1	7.1	16.7	69.0	n.s
Night 6						
Water	29	75.9	6.9	3.4	13.8	
Honey	36	52.8	13.9	8.3	25.0	n.s
Night 8						
Water	38	71.0	15.8	7.9	5.3	
Honey	38	26.3	39.5	7.9	26.3	$P < 0.05$

Table 3.6 The effect of adult food availability on flight duration (min) of *H. armigera* females on nights 1, 2, 4, 6 and 8.

		Percentage of moths flying				
	n	< 60	61 - 120	121 - 180	> 181	Chi-sq
Night 1						
Water	22	59.1	13.6	9.1	18.2	
Honey	22	45.5	9.1	13.6	31.8	n.s
Night 2						
Water	24	37.5	50.0	4.2	8.3	
Honey	22	45.5	18.2	9.1	27.2	n.s
Night 4						
Water	23	69.5	13.1	8.7	8.7	
Honey	22	27.3	18.2	0	54.5	$P < 0.05$
Night 6						
Water	22	77.3	13.6	9.1	0	
Honey	22	27.3	13.6	22.7	36.4	$P < 0.05$
Night 8						
Water	22	90.9	9.1	0	0	
Honey	22	40.9	13.6	18.2	27.3	$P < 0.05$

Table 3.7 Effects of mating and adult food availability on preoviposition period, lifetime fecundity and and longevity of *H. punctigera* and *H. armigera* adults. Values represent mean ± s.d. (range)

	n	Preoviposition period (days)	Fecundity (no. eggs / female)	Longevity (days)
H.punctigera				
Honey - mated	29	1.6 ± 1.2^{a} (0 - 5)	1619 ± 434^{a} (791 - 2665)	14.6 ± 2.4^{c} (6 - 18)
Honey - unmated	29	1.8 ± 1.1^{a} (0 - 4)	1120 ± 535^{b} (232 - 2084)	21.1 ± 3.8^{a} (15 - 30)
Water - unmated	29	1.5 ± 0.6^{a} (1 - 3)	703 ± 284^{c} (374 - 1348)	11.4 ± 2.3^{d} (7 - 15)
H.armigera				
Honey - mated	25	1.7 ± 0.6^{a} (0 - 3)	1440 ± 476^{a} (787 - 2532)	14.5 ± 2.2^{c} (10 - 18)
Honey - unmated	25	1.9 ± 0.8^{a} (1 - 3)	962 ± 439^{b} (138 - 1837)	18.1 ± 2.3^{b} (14 - 22)
Water - unmated	25	4.6 ± 1.0^{b} (3 - 7)	342 ± 217^{d} (106 - 946)	11.2 ± 2.4^{d} (7 - 14)

Values followed by differing superscript differ significantly at $P < 0.05$ (S.N.K test), (within column comparisons).

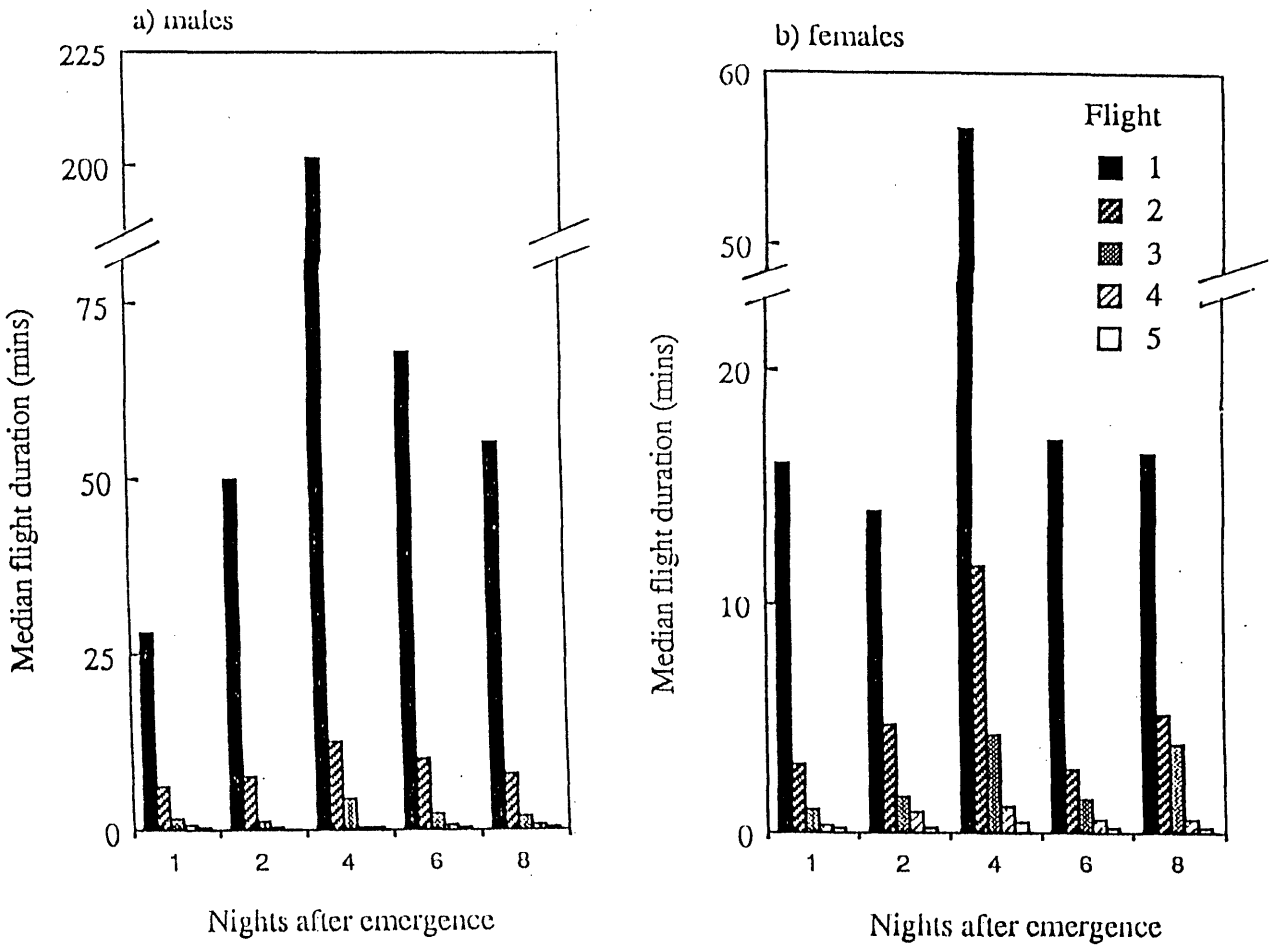

Fig 3.1 Median flight durations (mins) for *H. armigera* a) males and b) females completing 2 or more flights within the 5 hour test period on nights 1, 2, 4, 6, and 8. Flights ranked from the longest to the shortest duration on each night.

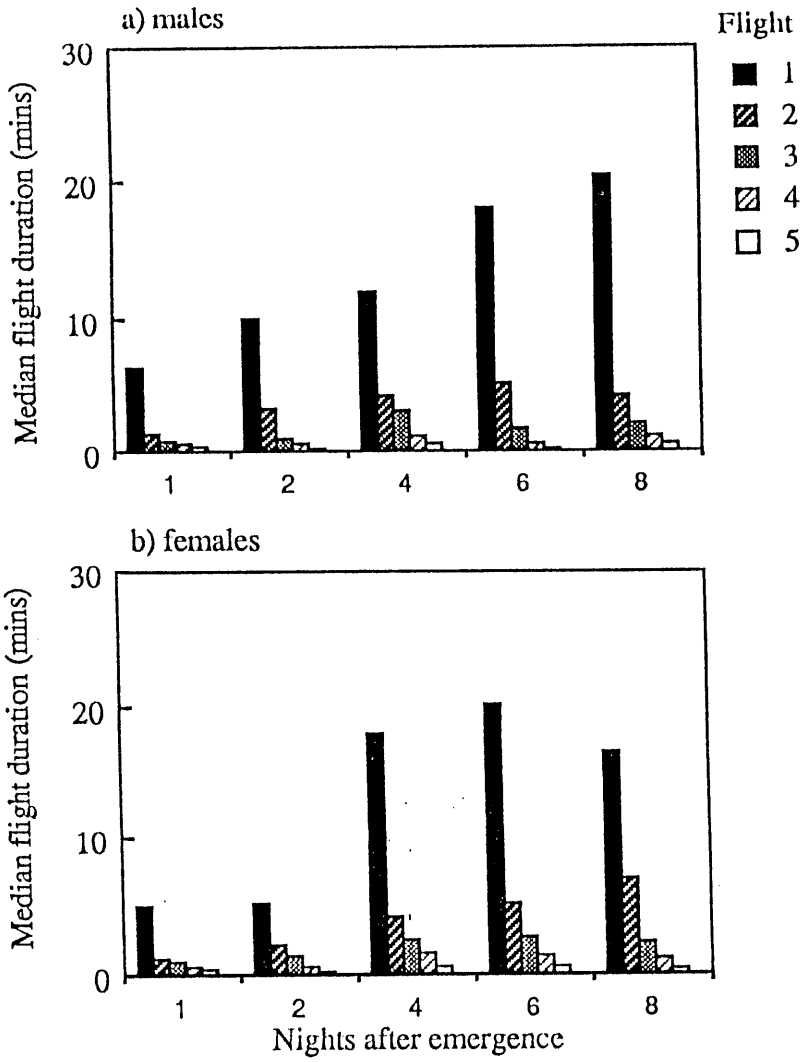

Fig 3.2 Median flight durations (mins) for *H. punctigera* a) males and b) females completing 2 or more flights within the 5 hour test period on nights 1, 2, 4, 6, and 8. Flights ranked from the longest to the shortest duration on each night.

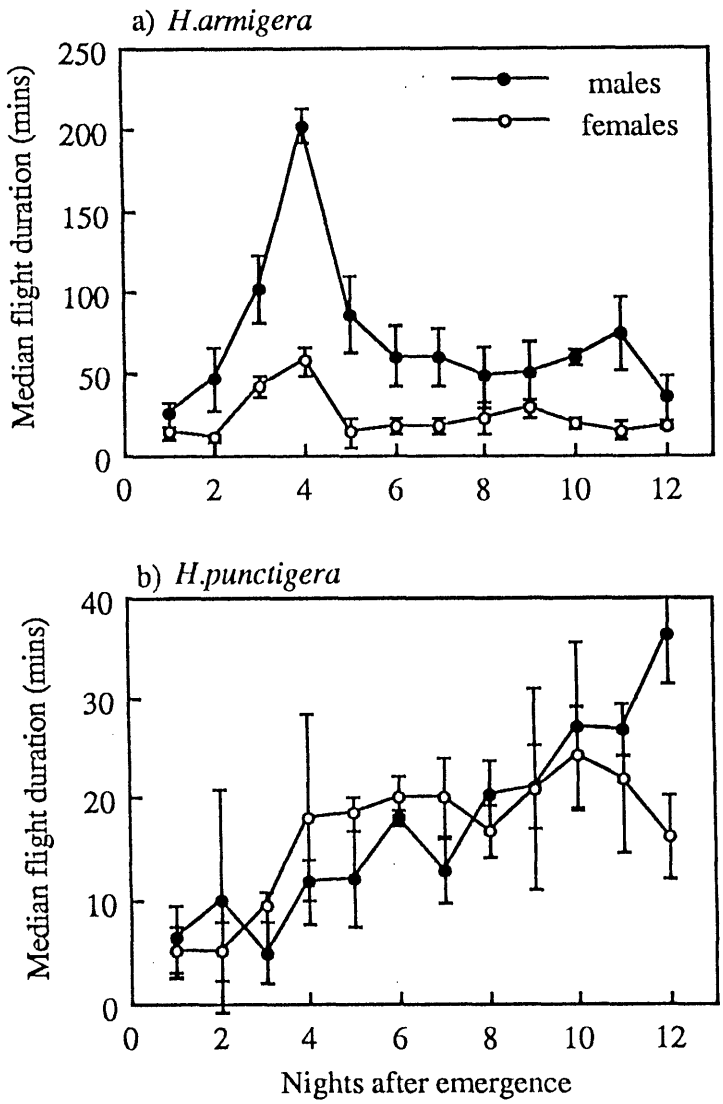

Fig 3.3 Median flight duration (min ± s.e) as a function of age for a) *H. armigera* females (n = 30) and males (n = 30) and b) *H. punctigera* females (n = 35) and males (n = 38).

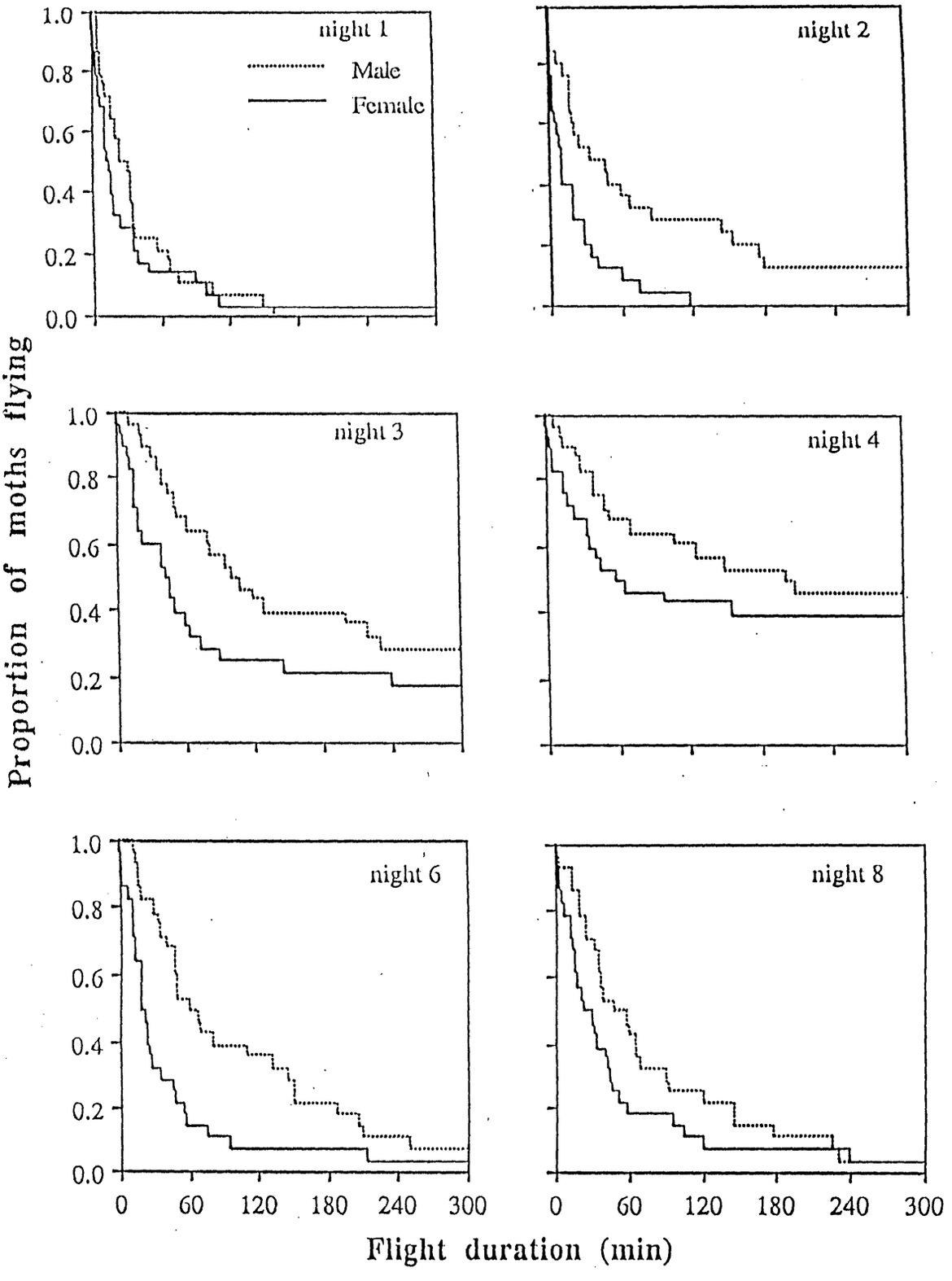

Fig 3.4 Flight distribution profiles for *H. armigera* females and males on nights 1 to 4, 6 and 8 after emergence.

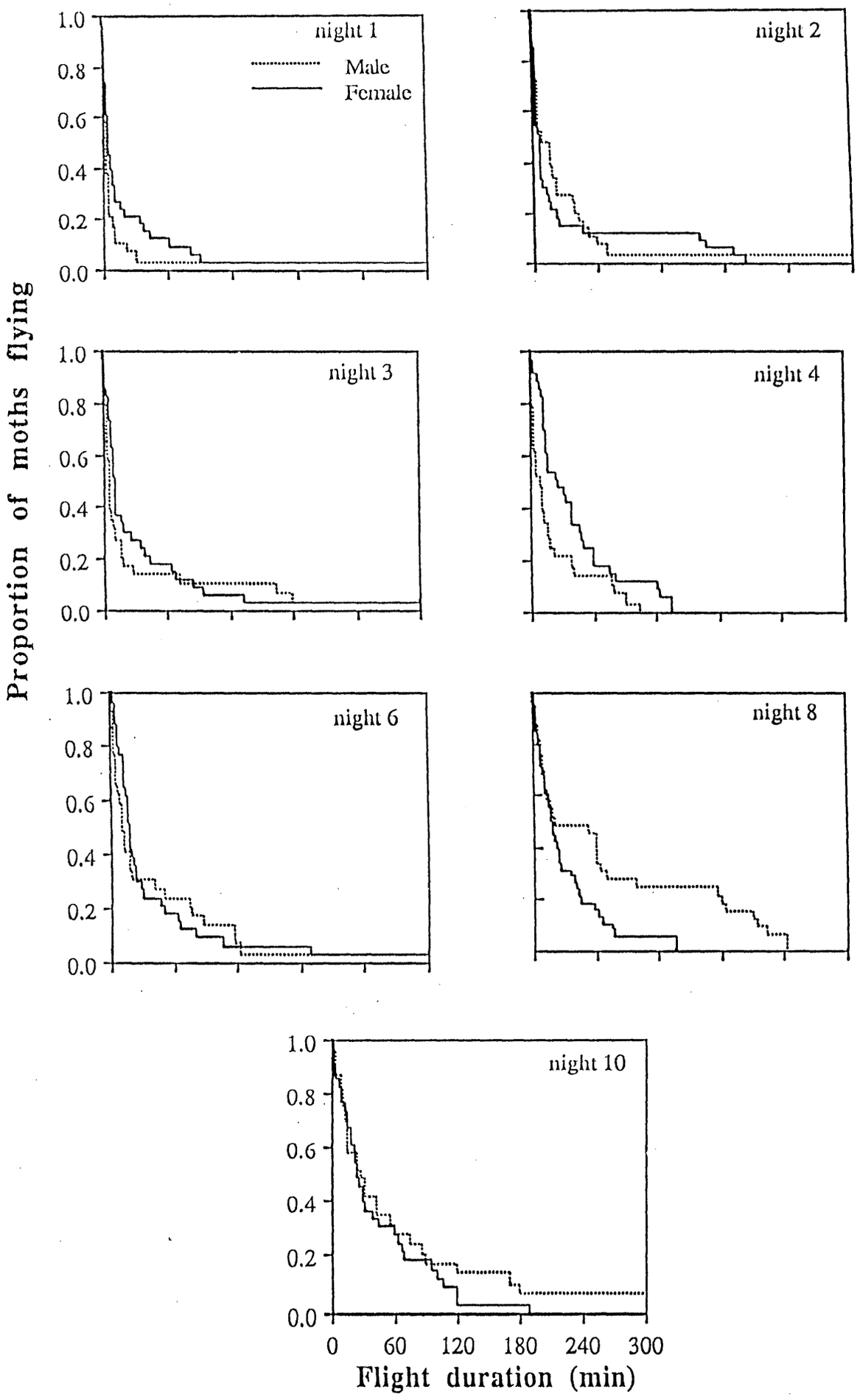

Fig 3.5 Flight distribution profiles for *H. punctigera* females and males on nights 1 to 4, 6, 8 and 10 after emergence.

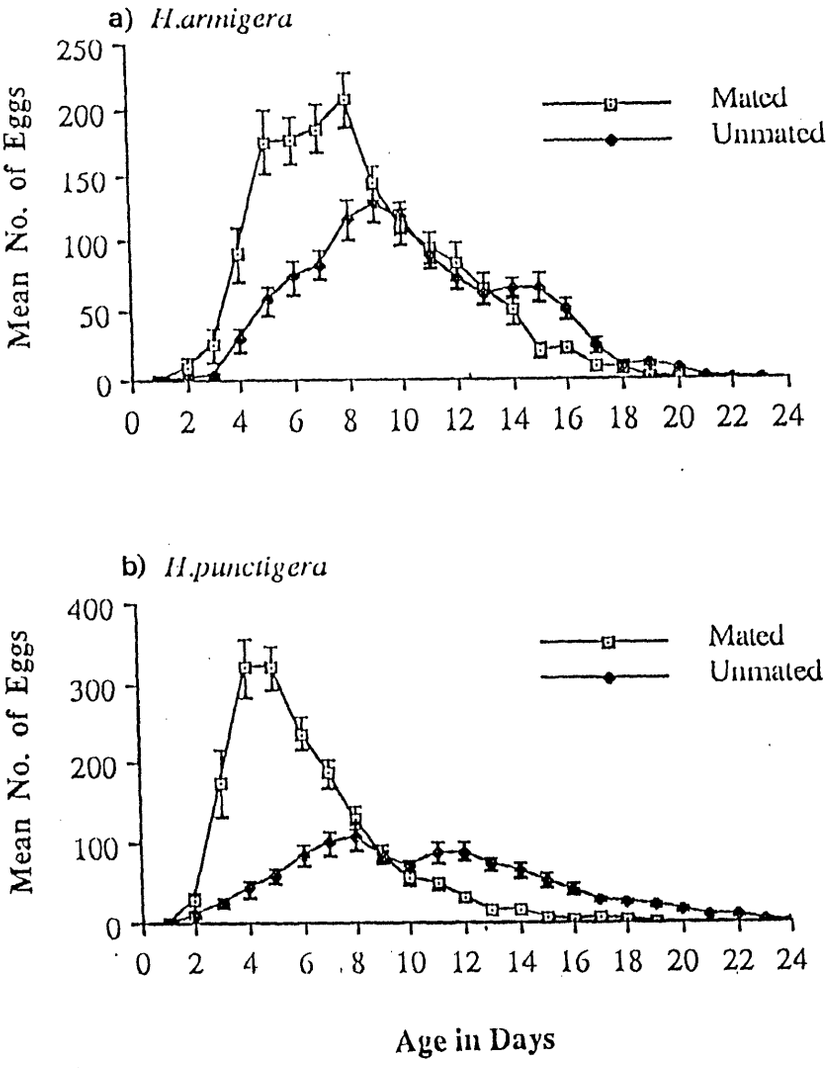

Fig 3.6 Oviposition profiles for mated and unmated for a) *H. armigera* females (n = 25) and b) *H. punctigera* females (n = 29).

CHAPTER 4
FLIGHT SPEED, DISTANCE AND MORPHOMETRICS

4.1 Introduction

For an insect moving within its flight boundary layer (*sensu* Taylor 1974), an important component of its ability to locate and travel between suitable habitat patches will be its flight speed. Biomechanical models of flight performance (Pennycuik 1972) predict that for geometrically similar animals, flight speed should increase as body mass increases. Further, bioenergetic models (Roff 1991) predict that flight distance, as a function of flight speed, availability of energy reserves, and metabolic rate, should also increase with body size. The aim of this chapter is to investigate the influence of body size on these flight parameters. The methodology employed in chapter 3 to examine the influence of age on flight performance was inappropriate to detect the effects of body size variables on flight parameters. A second tethered-flight methodology (flight mill) is employed here in attempting to identify possible body size correlates with flight ability. This methodology has the advantage that the insects are required to propel a horizontal arm in order to fly, allowing a comparative analysis of flight speed and distance flown.

4.2 Methods

4.2.1 Insect material

All moths used in the study represented 2nd or 3rd generation insects reared on artificial diet (Teakle & Jensen 1985). Cultures were derived from field collected eggs and larvae from the Armidale region of the New England Tablelands, New South Wales, Australia, during December 1989.

Larvae were maintained individually in 35 ml plastic cups at 25 ± 1°C and 60 - 70 % relative humidity in a 14:10 light dark regime. Pupation occurred in larval rearing containers amongst remaining diet and excess frass. Following pupation pupae were stored in groups of 10 - 12 individuals in 125 ml plastic cups. Prior to emergence

pharate pupae were transferred to and maintained individually in 285 ml plastic cups lined with paper towelling. Moths were supplied with 10 % honey solution from the time of emergence and this was renewed daily. Pupae and adults were maintained under the same conditions of photoperiod and temperature as those for rearing larvae.

4.2.2 Morphometric parameters

Three aspects of body size (body weight, body length and wing size) were examined as possible correlates with flight ability. Two measurements of body weight were recorded: pharate pupal weight (PPW) (mg) recorded on the afternoon prior to emergence, and body weight (BW) (mg) of the adult taken just prior to tethering. Pupal weight was found to decrease with increasing pupal age as shown in Fig. 4.1. Pupae were weighed as pharate adults as close as possible to the time of emergence, in order to reduce the influence of this variation. Two measurements of body length were recorded: pharate pupal length (PPL) (mm) and adult body length (BL) (mm). Two measurements of wing size were recorded: forewing wing length and area. Length of the forewing (FWL) (mm) was measured from the point of thoracic articulation to the wing apex. Wing area was determined by measuring the total area of one wing pair with a digital leaf area meter to the nearest millimeter, this value was then doubled to obtain gross wing area (GWA) (mm^2).

Adult body and forewing lengths were recorded following flight testing. Measurements of wing area involved removal of the fore- and hind-wings from the body. This was carried out after flight testing.

4.2.3 Flight muscle to body weight ratio

In order to examine the pssibility of a morphological basis for the increase in flight ability of *H. punctigera* and *H. armigera* adults, as observed in age-related tethered-flight (Ch. 3, Section 3.3.1), the relationship of thoracic muscle mass to body mass was determined for moths of age 1 and 4 days post-emergence. Live body weight (m_b) of adults was recorded to the nearest 0.1 mg using a digital balance.

Moths had previously been supplied with distilled water from the time of emergence as a food source. The head, abdomen, and appendages were excised and the thorax cut sagitally to remove the oesophagus. Thoracic mass (m_t) was determined by weighing the two halves of the thorax to the nearest 0.1 mg. The thorax was then soaked for 24 hr in NaOH solution (0.36M). This removed the soft tissues leaving the thoracic exoskeleton. The thoracic exoskeleton was removed, washed in distilled water and dried in an oven at 60 °C; it was periodically weighed (m_{exo}) until no further weight change was recorded. The difference between m_t and m_{exo} was taken as the mass of the flight musculature (m_{fm}). This measurement is confounded to some extent by the musculature associated with the legs; however, this is considered negligible in relation to the mass of the flight apparatus (Chapman 1982). From these measurements the ratio of flight muscle mass to body mass (FMR) was calculated.

4.2.4 Flight mill

The flight mill consisted of a horizontal arm (circumference = 1.0 m, diameter = 2.0 mm) supported by a vertical rod (diameter = 2.0 mm) which rotated freely between two hollow tipped screws. Both the upper and lower ends of the vertical rod were finely pointed and rested within the hollow of the supporting screw, and acted as the bearing surface for the mill arm. A paper flag attached to the mill arm triggered an optical switch to record each revolution of the mill arm. An electronic data logger computed the total distance travelled.

4.2.5 Flight testing

Moths were flown on nights 1 or 4 post emergence and were unmated. On the afternoon prior to flight testing moths were anaesthetised once using diethyl ether and a 5 mm length of polyethylene tubing was attached to the dorsal surface of the thorax with contact adhesive. The tubing provided a friction fit onto a downward pointing tip of the mill arm. Moths were allowed to recover from anaesthetisation for a minimum period of 2 h prior to flight testing. Flights were initiated, by forced loss of tarsal

contact, at the commencement of the dark phase. Moths were flight tested for a period of 10 h. Flight speed (km hr $^{-1}$) was recorded at 15 min intervals during an initial period of 90 min for 1 day old moths, and 180 min for 4 day old moths, as an average for the previous 15 minute period. Moths remained attached to the mill arm for the remainder of the 10 hours. Total flight distance was recorded at the completion of the 10 h period. Individual moths were discarded after one flight test.

4.3 Results

4.3.1 Morphometrics

Considerable variation was observed in adult body weight on the day following emergence. Adult body weights at this time were more variable than expected given the observed variation in pharate pupal weights; this variability was found to be the result of variation in the time taken to expel the pupal meconium. The relationship between PPW and BW on the day following emergence is shown for *H. punctigera* in Fig 4.2. Data are presented for a sample of males (Fig 4.2a) and females (Fig 4.2b) that had either fully retained or which had either partly or entirely expelled the meconium. Expulsion of the meconium was marked by the release of a viscous brown liquid, which was readily observable in holding containers. Variability in the time taken to expel the meconium and its resultant influence on adult body weight (BW) at this time provides sufficient grounds to reject this parameter as a reliable measurement of body size. Consequently pharate pupal weight (PPW) was used as the sole measurement of body weight. Pharate pupal length (PPL) and body length (BL) were found to be closely correlated. This was true for *H. punctigera* (males: $r^2 = 0.86$, $F = 289.6$, $P < 0.001$; females: $r^2 = 0.5$, $F = 50.9$, $P < 0.001$) and *H. armigera* (males: $r^2 = 0.71$, $F = 128.4$, $P < 0.001$; females: $r^2 = 0.87$, $F = 346.3$, $P < 0.001$).

Gross wing area GWA was found to be closely correlated with forewing length (FWL) in *H. punctigera* and *H. armigera* adults. Regressions of GWA on FWL were significant for *H. armigera* females ($b = -363$, $a = 39.0$, $r^2 = 0.84$, $F_{1,18} = 99.4$, $P < 0.001$) and males ($b = -207$, $a = 30.7$, $r^2 = 0.81$, $F_{1,23} = 97.2$, $P < 0.001$) and *H.*

punctigera females (b = -410, a = 45.5, r^2 = 0.90, $F_{1,16}$ = 158.6, P < 0.001)) and males (b = -315, a = 39.4, r^2 = 0.85, $F_{1,18}$ = 108.5, P < 0.001). Plots of GWA versus FWL are shown in Fig 4.3a for *H.armigera* males and females and Fig 4.3b for *H.punctigera* males and females. The close correlation between GWA and FWL suggest that FWL can be used as the sole measurement of wing size. This removed the necessity of killing moths following flight testing and eliminated the lengthy time involved in determining wing area. As BW is proportional to PPW (Fig. 4.2) and GWA is proportional to FWL (Fig 4.3), wing loading, the relationship between body weight and wing area, was estimated from the ratio of PPW to FWL.

Data for morphometric measurements on 106 *H.punctigera* and 112 *H.armigera* are presented in Table 4.1. Analysis of variance of these measurements indicated that there were significant differences between species and significant sex differences within species with respect to each parameter. Significant differences were detected between PPW ($F_{3,215}$ = 184.4, P < 0.001), PPL ($F_{3,215}$ = 148.7, P < 0.001), FWL ($F_{3,215}$ = 240.6, P < 0.001) and BL ($F_{3,215}$ = 125.9) and the ratio PPW / FWL ($F_{3,215}$ = 66.0, P < 0.001). Following analysis of variance significant differences were identified using a multiple comparison procedure (SNK test) (Zar 1974). In each of the parameters measured *H.armigera* adults were significantly larger than *H.punctigera* adults (P < 0.05 in each case, SNK-test). Intra-specific sex differences were also evident for some parameters. In both *H.punctigera* and *H.armigera,* males were significantly longer than females as indicated by both PPL and BL (P < 0.05 SNK test), though PPW and FWL did not differ significantly between sexes in either species (P > 0.05 SNK-test). There was no intra-specific sex difference for the ratio of PPW to FWL (P > 0.05 SNK-test), however, the ratio was significantly higher (P < 0.05, SNK-test) for *H.armigera* than *H.punctigera* moths.

4.3.2 Flight speed and distance

Mean flight speeds (km hr^{-1}) on night 1, averaged over 15 min intervals for a period of 90 min, for *H.armigera* males and females and *H.punctigera* males and

females are shown in Fig 4.4a and b respectively. All moths, whether *H.punctigera* or *H.armigera,* flew continuously throughout the 90 min observation period. No moths stopped flying during this period. Casual observation of tethered moths indicated that on occassions some moths ceased flying shortly after this 90 min observation period. Total flight distance for these moths was noted at this time and found not to differ greatly from the total flight distance achieved overnight, indicating that these moths undertook little further flight. Moths while remaining attached to the flight mill arm, were generally not observed again until completion of the 10 hr flight test period. On night 1 there was an initial trend for flight speed to decrease with increasing flight time for both species. Flight speed then either levelled or rose slightly. Repeated measures analysis of variance (Dixon 1981) indicated significant differences in flight speed between species ($F_{1,84} = 28.4$, $P < 0.001$), but not intraspecific sex differences ($F_{1,84} = 0.15$, $P > 0.5$). Significant linear ($F_{1,84} = 39.1$, $P < 0.001$), quadratic ($F_{1,84} = 46.9$, $P < 0.001$) and cubic ($F_{1,84} = 7.3$, $P < 0.01$) components were identified, reflecting the curvilinear change in flight speed observed with increasing flight time. Mean flights speeds during the final 15 min of the 90 min test period were 1.5 ± 1.5 and 1.3 ± 1.1 km hr^{-1} for *H.punctigera* males and females and 3.0 ± 1.1 and 3.6 ± 1.4 km hr^{-1} for *H.armigera* males and females.

Mean flight speeds (km hr^{-1}) on night 4, similarly averaged over 15 min intervals but for a period of 180 min, for *H.armigera* males and females and *H.punctigera* males and females are shown in Fig 4.5a and b. As occurred on night 1, all moths, whether *H.punctigera* or *H.armigera,* were observed to fly continuously throughout the 180 min observation period. No moths ceased flying prior to the completion of this period. Repeated measures analysis of variance indicated a significant difference between species ($F_{1,102} = 23.4$, $P < 0.001$), but no sex difference ($F_{1,102} = 0.35$, $P > 0.5$) within species. No significant time effect was observed ($F_{1,102} = 0.88$, $P > 0.3$), indicating that flight speed remained relatively constant over the 180 min period during which measurements were recorded. Mean flight speeds during the final 15 min of the 180 min test period were 3.6 ± 1.5 and 3.4

± 1.5 km hr $^{-1}$ for *H. punctigera* males and females and 4.8 and 5.2 km hr $^{-1}$ for *H. armigera* males and females respectively.

Flight distances achieved during 10 h of tethered-flight by *H. punctigera* males and females and *H. armigera* males and females are shown in Table 4.2. Analysis of variance of flight distances indicated significant differences between groups on night 1 ($F_{3,105} = 10.4$, $P < 0.001$) and night 4 ($F_{3,117} = 4.65$, $P < 0.01$). Multiple comparison procedures (SNK test) were performed to identify these differences. Data for moths flown on night 1 and night 4 were analysed separately. Numbers of individual flights undertaken during the 10 hr test period were not recorded, other than the observation that on nights 1 and 4 moths flew continuously throughout the observation periods (90 and 180 min respectively).

On night 1, mean flight distances of 10.0 km and 12.1 km were recorded for *H. punctigera* males and females, and 28.7 km and 26.1 km for *H. armigera* males and females respectively. Mean flight distances of males and females did not differ significantly within respective species ($P > 0.05$, SNK-test); however, mean flight distances of *H. armigera* adults were significantly greater than those of *H. punctigera* adults ($P < 0.05$, SNK-test). On night 4, mean flight distances of 35.6 km and 29.2 km were recorded for *H. punctigera* males and females and 42.9 km and 33.7 km for *H. armigera* males and females respectively. Mean flight distances of males and females within their respective species did not differ significantly ($P > 0.05$, SNK-test). There was, however, no clear difference in mean flight distance achieved between species. Mean flight distance of *H. punctigera* males did not differ significantly from those of *H. armigera* males or females (SNK-test, $P > 0.05$) though mean flight distance of *H. punctigera* females was significantly less than those of *H. armigera* males and females (SNK-test, $P < 0.05$). Though not compared directly, mean flight distances achieved during 10 hr of flight increased from night 1 to night 4 for both species. This is consistent with increasing flight ability with age as determined in Chapter 3.

Maximum flight distances recorded on night 1 were 62.9 km and 52.3 km for *H. punctigera* males and females and 60.5 km and 59.2 km for *H. armigera* males and females respectively. On night 4, maximum flight distances recorded were 75.4 km and 64.1 km for *H. punctigera* males and females and 69.2 km and 62.8 km for *H. armigera* males and females. In some instances moths flown overnight, while achieving significant flight distances, exhibited signs of exhaustion, being either unable to respond to stimuli appropriate to initiate flight or if capable of initiating flight only flying weakly. In some instances long flying moths were dead on the morning following flight testing or died shortly after being removed from the mill arm (approx 10 % of moths flight tested). All moths were supplied with 10 % honey solution both immediately prior to and after flight testing. These results suggest that moths may fly to exhaustion while tethered to the mill arm. The minimum lifespan (days) for unflown *H.punctigera* and *H.armigera* moths is 15 and 14 days (see Table 3.7).

Comparison of mean flight speeds exhibited during the observation period and distances achieved overnight suggest that a high proportion of both *H. punctigera* and *H. armigera* moths must have flown continuously throughout the test period in order to achieve the observed magnitude of flight distances.

4.3.3 Flight distance and morphometrics

Flight distance did not correlate with PPW or FWL in either species on nights 1 or 4 (r^2 values were consistently less than 0.1 in all regressions) (see Tables 4.3 and 4.4). Figs 4.6a and b show plots of flight distance against PPW and Figs 4.7a and b plots of flight distance against FWL for *H. armigera* on these nights. Figs 4.8a and b and 4.9a and b show similar plots of flight distance against PPW and FWL for *H. punctigera* moths. Similarly, the ratio of PPW to FWL, though significantly different between species, did not correlate with distance flown within species (r^2 values consistently less than 0.1 in all regressions) (see Tables 4.3 and 4.4).

4.3.4 Flight muscle to body weight ratio in *H.punctigera*

Table 4.5 shows the ratio of flight muscle mass (m_{fm}) to body mass (m_b) (= flight muscle ratio: FMR) for *H. punctigera* males and females at 1 and 4 days of age post emergence. Insufficient adults were available from cultures to undertake these measurments for *H. armigera*. For *H. punctigera* females a significant increase in both m_{fm} ($t_{42} = -2.24$, $P < 0.05$) and m_b ($t_{42} = -4.97$, $P < 0.001$) occurred between days 1 and 4 post emergence. In males, however, neither m_{fm} ($t_{35} = -0.27$, $P > 0.5$) or m_b ($t_{35} = 0.75$, $P > 0.25$) differed significantly between the ages of 1 and 4 days. The calculated FMR ratio decreased significantly ($t_{42} = 5.00$, $P < 0.001$) for *H. punctigera* females during this period, but remained unchanged for *H. punctigera* males ($t_{35} = -1.63$, $P > 0.1$).

4.4 Discussion

Comparison of flight speeds and flight distances exhibited during 10 h of tethered-flight identified significant differences between *H. armigera* and *H. punctigera* adults. On night 1, *H. armigera* males and females exhibited significantly greater mean overnight (10 h) flight distances than *H. punctigera* adults. On night 4, however, mean flight distances of *H. punctigera* males did not differ significantly from those of *H. armigera* females and males, being intermediate between the two. Mean flight distance of *H. punctigera* females only differed significantly from that of *H. armigera* males. Comparison of flight speeds exhibited during the first 3 h of flight similarly identified *H. armigera* as exhibiting significantly greater flight speeds than *H. punctigera* moths. Though no correlation was found between flight performance and any feature of body size within species (see below) the observed differences between the two species may be due to differences in their respective body sizes. *H. armigera* adults were on average significantly larger than *H. punctigera* adults in regard to each of the morphometric parameters measured during this study. Increased body size

obviously confers some advantage in overcoming the inertia and frictional drag of the mill arm.

The observed increase in mean flight distances and flight speeds for both species between nights 1 and 4 are consistent with the results of age-related flight profiles generated from static tethered-flight (Chapter 3). Median flight durations (mins) were shown to increase in both *H. punctigera* and *H. armigera* to peak during early adult life. The results here show that flight speed and flight distance, as a function of speed and duration, similarly increase during this period.

Tethered-flight speeds of *H. armigera* adults shown here are comparable with the results of Armes & Cooter (1991) for *H. armigera* from India. Armes & Cooter demonstrated mean flight speeds of between 3.35 and 4.84 km hr $^{-1}$. How these speeds relate to free flight under natural conditions has not been determined. Based on radar studies, Farrow & Daly (1987) give flight speeds of 14 km hr $^{-1}$ for *Helicoverpa* adults under natural conditions. Clearly flight speeds generated during tethered-flight studies such as obtained here and by Armes and Cooter provide only relative estimates of flight ability between sexes and species; they should not be interpreted as absolute measures of flight ability. The insects, under these experimental conditions, in addition to the drag imposed by their own bodies, must overcome the frictional drag of the mill arm, while being limited to a circular flight path. It is logical to suppose that these factors operate to restrict the realised flight speed of the insects on the mill.

Distributions of flight performance during static tethered-flight were characterised by a predominance of short fliers (see Section 3.3.1). This is consistent with, and typical of, the findings of similar studies (Johnson 1969, Davis 1980, Naranjo 1990). Distributions of overnight flight distances generated on the flight mill, however, did not exhibit a skew towards short flights, other than for *H. punctigera* adults on night 1. Armes & Cooter (1991) similarly found that flight distributions of *H. armigera* adults were either normally distributed or skewed towards long flights rather than sharply skewed towards short flights. They similarly employed a flight mill to assess flight ability. This discrepancy suggests possible differences in the way that

moths respond to the two tethered-flight methodologies. It is clear from comparison of overnight flight distances and average flight speeds, at least on night 4, that the majority of individuals of both species must have flown throughout the 10 h test period. This contrasts with the results of the static tethered-flight assays (Chapter 3), where the majority of adults undertook flights of less than 1 h. The results presented here suggest that moths flown on a flight mill respond by flying continuously and in some instances to exhaustion, possibly as a consequence of the increased sensory inputs presented. Dingle (1985) has suggested that a number of techniques should be employed to assess flight performance as any one method may either fail to reveal components or provide misleading results. It is clear from the results provided here that static tethered-flight and flight on a mill produce differing results with respect to flight duration. The two methods, however, both identify firstly a phase of increasing flight ability during early adult life and secondly significant differences in flight ability between the two species.

No correlation between body size parameters and flight ability were detected in either species. Biomechanical (Pennycuik 1972) and bioenergetic (Roff 1991) theories predict the flight speed and flight distance of large individuals to be greater than that of small individuals, given similar body proportions. No such relationship was evident for the measurements of body size employed during this study, other than differences in mean flight speed and distance being inferred from differences in overall body size between *H. armigera* and *H. punctigera*. Despite these findings there are a number of studies involving field (Danthanarayana 1976) and genetic (Dingle & Evans 1987) evidence that infer size related influences on flight ability. Direct measurements of flight speed in the field (DeVries & Dudley 1990) and tethered-flight studies (Davis 1980, Naranjo 1990), however, have not shown any correlation between morphometric characteristics and quantitative measurements of flight performance.

Studies of age-related flight performance (Chapter 3) and the comparison of flight speeds and over-night flight distances (this chapter) identify a phase of increasing flight ability during early adult life in *H. punctigera* and *H. armigera*. The possibility of a morphological basis for this observation was examined by comparing age-related

changes in body weight relative to flight muscle weight in *H. punctigera* adults. A number of findings are of potential significance. Firstly, females were observed to undergo an increase in mean body mass (m_b) between the ages of 1 and 4 days post emergence, whereas mean m_b of males remained unchanged. As mean wing length does not change with age, this indicates that wingloading, as a function of wing area and body weight, increases in females but remains unchanged in males between nights 1 and 4. Secondly, in females there was a significant increase in the mass of the flight musculature (wet mass) during this period; however, no increase was recorded in males. This suggests that the increase in mean flight ability during early adult life recorded for both male and female *H. punctigera* and *H. armigera* cannot be explained by changes in the mass of the flight musculature (m_{fm}). The observed increase in flight muscle weight in females may, therefore, be a response to the increased load imposed by weight gain resulting from egg maturation. Males undergo no such weight gain, and do not display any concomitant gain in m_{fm}. Despite the accompanying increase in m_{fm} in females between nights 1 and 4 the calculated ratios of m_{fm} to m_b (FMR) declined significantly during this period. This ratio provides an estimate of the load carried by the flight muscles, in this case adult body weight (Marden 1989). This shows that despite the accompanying increase in flight muscle weight the constraints of load lifting increase for females during early adult life. Other morphological changes that may have occurred, but which were not examined, include increases in mitochondrial density of the flight muscles (Chapman 1982).

Summary

Significant differences in mean flight speed and mean flight distance during a 10 hour period of tethered-flight were detected between *H. punctigera* and *H. armigera.* These measures of flight capacity were found to increase between nights 1 and 4 post emergence in both species. These results are consistent with the findings of static tethered-flight examined in Chapter 3. *H. armigera* displayed significantly greater

flight speeds and over-night flight distances than *H. punctigera* on night 1, however, differences in mean flight distance were not as evident on night 4.

Though *H. armigera* adults are significantly larger than *H. punctigera* with respect to each of the body size parameters measured, no correlation between any measure of body size and quantitative measures of flight ability were detected.

H. punctigera females exhibit an increase in flight muscle weight (wet) between nights 1 and 4. It is suggested that this increase, concomittant with an increase in body weight arising as a consequence of maturation of the ovaries, is a response by the female to counter increasing wingloading experienced during this period. No such increase in either body weight or flight muscle weight was detected in *H. punctigera* males.

4.5 References

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Table **4.1** Morphometric variables of *H. punctigera* and *H. armigera* adults. Values represent mean ± s.d. Pharate pupal weight: PPW, pharate pupal length: PPL, forewing length: FWL, body length: BL.

			Parameter				
		n	PPW (mg)	PPL (mm)	FWL (mm)	BL (mm)	PPW / FWL
H.punctigera	male	56	$292.3^{a} \pm 19.4$	$18.6^{b} \pm 0.7$	$15.6^{a} \pm 0.5$	$18.7^{b} \pm 0.7$	$18.7^{a} \pm 1.3$
	female	50	$278.1^{a} \pm 32.1$	$18.0^{a} \pm 0.5$	$15.8^{a} \pm 0.6$	$18.1^{a} \pm 0.6$	$17.6^{a} \pm 2.2$
H.armigera	male	48	$398.9^{b} \pm 35.6$	$20.9^{d} \pm 0.9$	$18.6^{b} \pm 0.9$	$21.0^{d} \pm 1.1$	$21.4^{b} \pm 1.9$
	female	64	$402.1^{b} \pm 58.3$	$20.4^{c} \pm 1.3$	$18.8^{b} \pm 1.2$	$20.2^{c} \pm 1.4$	$21.5^{b} \pm 2.5$

Values followed by differing superscripts differ significantly at $P < 0.05$ (SNK test). (Within column comparisons).

Table 4.2 Average distances achieved (km) during 10 hr of tethered-flight by virgin *H. punctigera* and *H. armigera* adults on nights 1 and 4 post emergence.

	Night 1				Night 4			
	n	x	s.e	range	n	x	s.e	range
H. punctigera								
male	28	10.0[a]	2.4	0.4 - 62.9	19	35.6[ab]	4.6	8.0 - 75.4
female	25	12.1[a]	2.7	1.6 - 52.3	37	29.2[b]	2.6	5.4 - 64.1
H. armigera								
male	29	28.7[b]	3.1	2.4 - 60.5	36	42.9[a]	2.3	13.3 - 69.2
female	27	26.1[b]	3.6	0.9 - 59.2	29	33.7[ab]	2.8	5.9 - 62.8

Values followed by differing superscripts differ significantly at $P < 0.05$ (SNK test). (Within column comparisons)

Table **4.3** Analysis of variance and regression statistics for relationships between morphometric variables (PPW, FWL and PPW / FWL) and flight distance for *H. punctigera* and *H. armigera* on night 1. PPW: pharate pupal weight (mg), FWL: forewing length (mm), PPW / FWL: estimate of wing loading.

	n	slope	y-intercept	r^2	F	P
Distance vs. PPW						
H. punctigera males	27	0.12	-26.9	0.07	1.67	n.s
females	24	-0.23	76.7	0.11	3.92	n.s
H. armigera males	29	-0.13	84.1	0.02	1.69	n.s
females	27	0.11	-19.6	0.10	4.06	n.s
Distance vs. FWL						
H. punctigera males	27	0.01	13.0	0.11	4.21	P = 0.05
females	24	-5.51	98.4	0.02	1.49	n.s
H. armigera males	29	-5.45	132.0	0.01	1.17	n.s
females	27	4.37	-57.2	0.04	2.10	n.s
Distance vs. PPW / FWL						
H. punctigera males	27	0.92	-7.5	0.01	0.30	n.s.
females	24	-1.88	45.7	0.05	1.32	n.s.
H. armigera males	29	-2.03	74.0	0.03	0.82	n.s.
females	27	2.42	-27.8	0.11	3.32	n.s.

Table 4.4 Analysis of variance and regression statistics for relationships between morphometric variables (PPW, FWL and PPW / FWL) and flight distance for *H. punctigera* and *H. armigera* on night 4. PPW: pharate pupal weight (mg), FWL: forewing length (mm), PPW / FWL: estimate of wing loading.

		n	slope	y-intercept	r^2	F	P
Distance vs. PPW							
H. punctigera	males	22	-0.31	128.0	0.05	1.02	n.s.
	females	35	-0.12	60.1	0.06	2.00	n.s.
H. armigera	males	28	0.01	41.9	0.00	0.01	n.s.
	females	22	-0.06	58.5	0.03	0.66	n.s.
Distance vs. FWL							
H. punctigera	males	22	-17.4	307.0	0.17	3.99	n.s.
	females	35	-15.9	281.0	0.13	5.04	n.s.
H. armigera	males	28	-0.32	50.4	0.00	0.01	n.s.
	females	22	3.72	-33.7	0.09	2.09	n.s.
Distance vs. PPW / FWL							
H. punctigera	males	22	0.64	22.4	0.01	0.02	n.s.
	females	35	-1.36	51.0	0.03	1.00	n.s.
H. armigera	males	28	0.12	42.0	0.00	0.01	n.s.
	females	22	-2.40	83.9	0.08	3.02	n.s.

Table 4.5 Flight muscle to body weight ratio (FMR) of male and female *H. punctigera* on nights 1 and 4 after emergence. m_b: body mass, m_{fm}: mass of flight musculature.

	Male			Female		
	m_b (mg)	m_{fm} (mg)	FMR	m_b (mg)	m_{fm} (mg)	FMR
Night 1	155 ± 8.0^a	40.0 ± 1.2^a	0.263^a	172.4 ± 6.6^a	40.5 ± 0.8^a	0.238^a
Night 4	147 ± 6.6^a	40.5 ± 1.0^a	0.279^a	221.6 ± 7.4^b	42.7 ± 0.5^b	0.197^b

Values represent mean ± s.d. Values following by differing superscripts differ significantly at $P < 0.05$ (t - test)

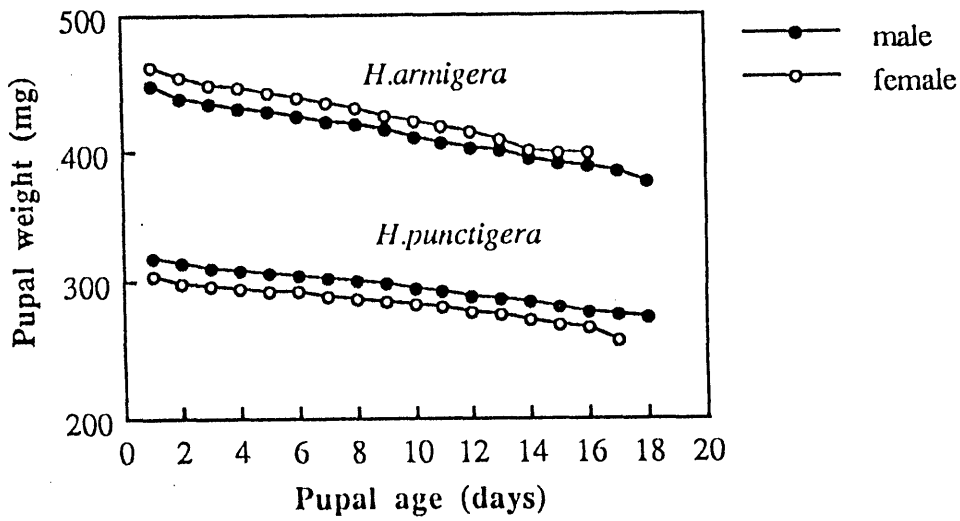

Fig 4.1 The relationship between pupal weight (mg) and pupal age (days since pupation) for *H. punctigera* and *H. armigera.*

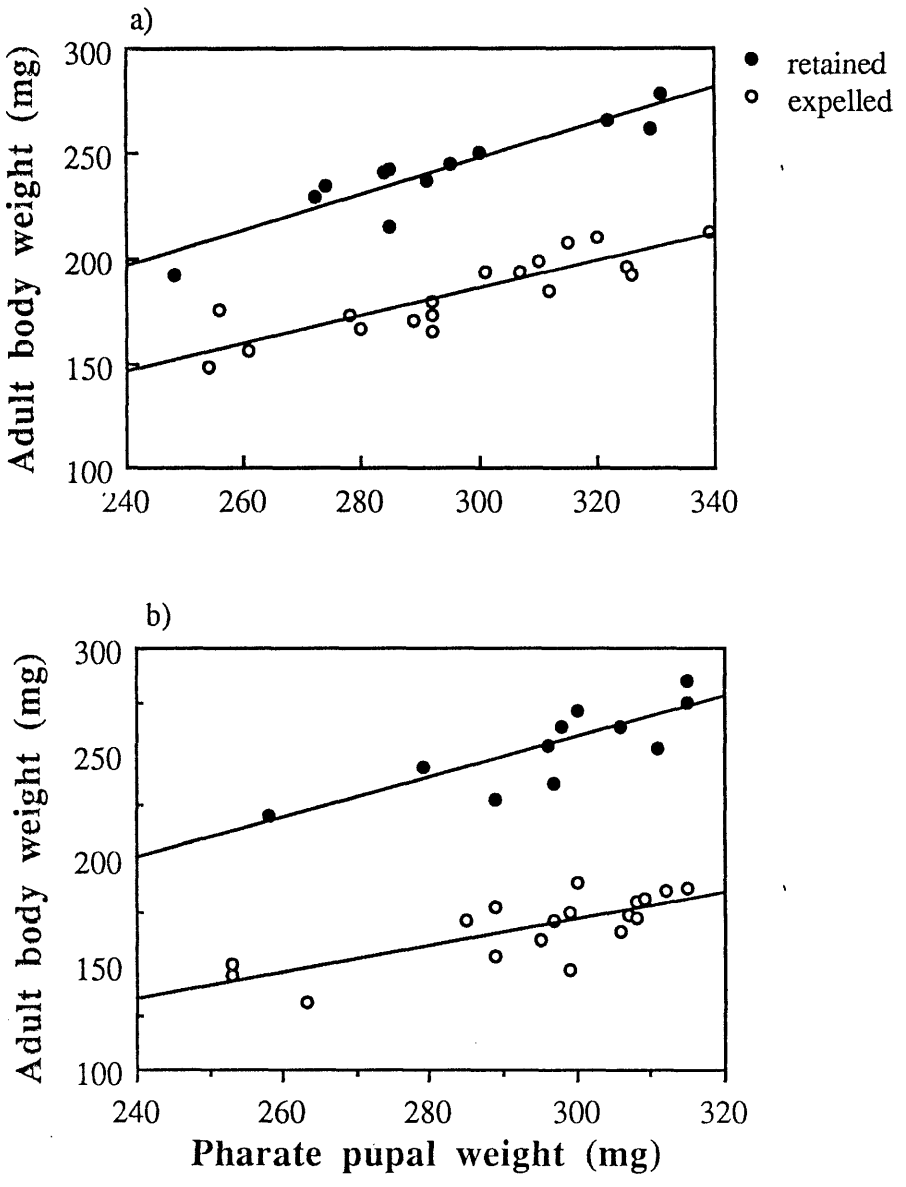

Fig 4.2 The relationship between pharate pupal weight (mg) and adult body weight (mg) on the day after emergence for *H. punctigera* a) males and b) females, which had either retained or expelled the pupal meconium.

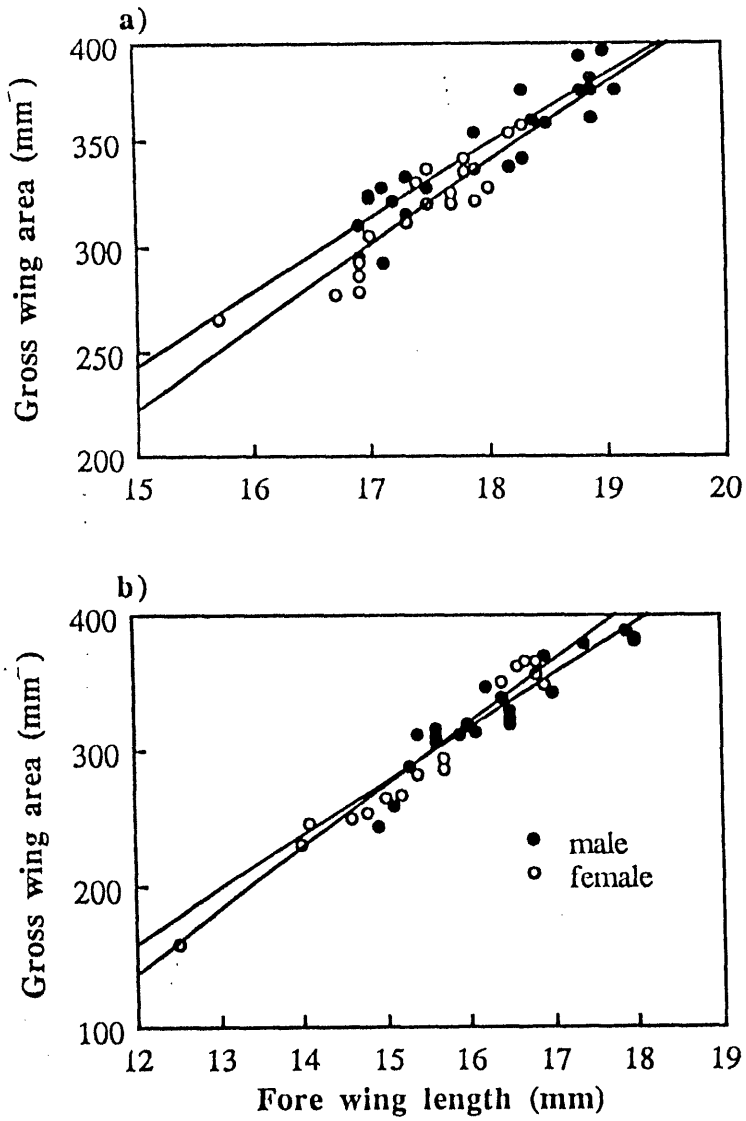

Fig 4.3 The relationship of gross wing area (GWA) (mm^2) to forewing length (FWL) (mm) for a) *H. armigera* males and females, and b) *H. punctigera* males and females.

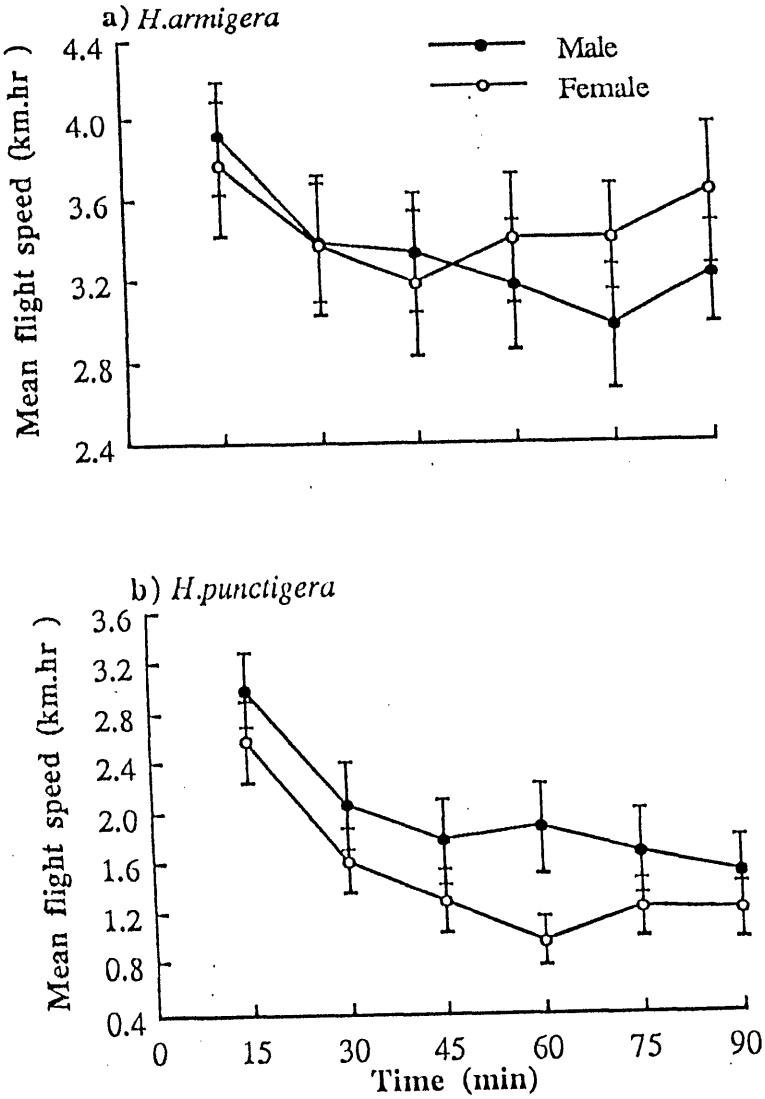

Fig 4.4 The relationship between flight speed (km hr^{-1}) and flight time (min) for a) *H. armigera* males and females, and b) *H. punctigera* males and females on the night following emergence. Flight speeds represent 15 min averages. Standard deviations given as vertical bars.

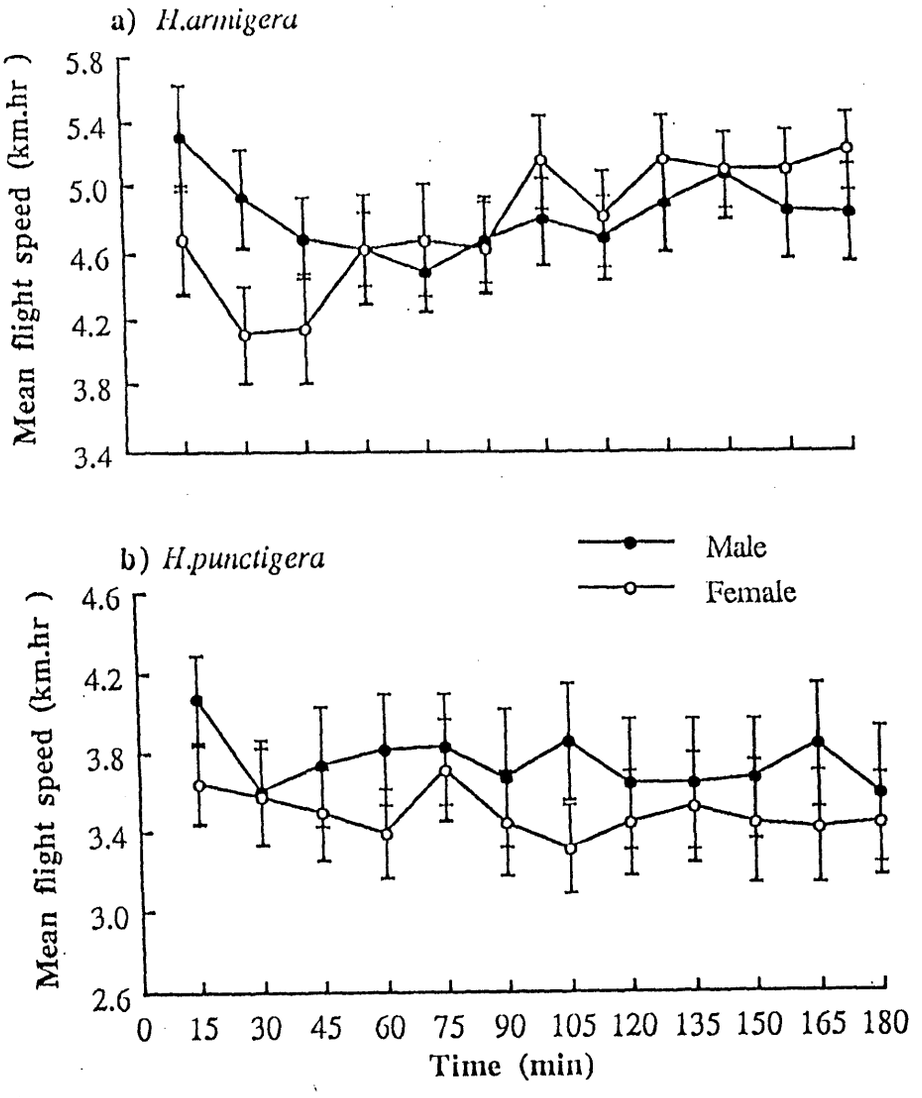

Fig 4.5 The relationship between flight speed (km hr^{-1}) and flight time (min) for a) *H. armigera* males and females, and b) *H. punctigera* males and females on night 4. Flight speeds represent 15 min averages. Standard deviations given as vertical bars.

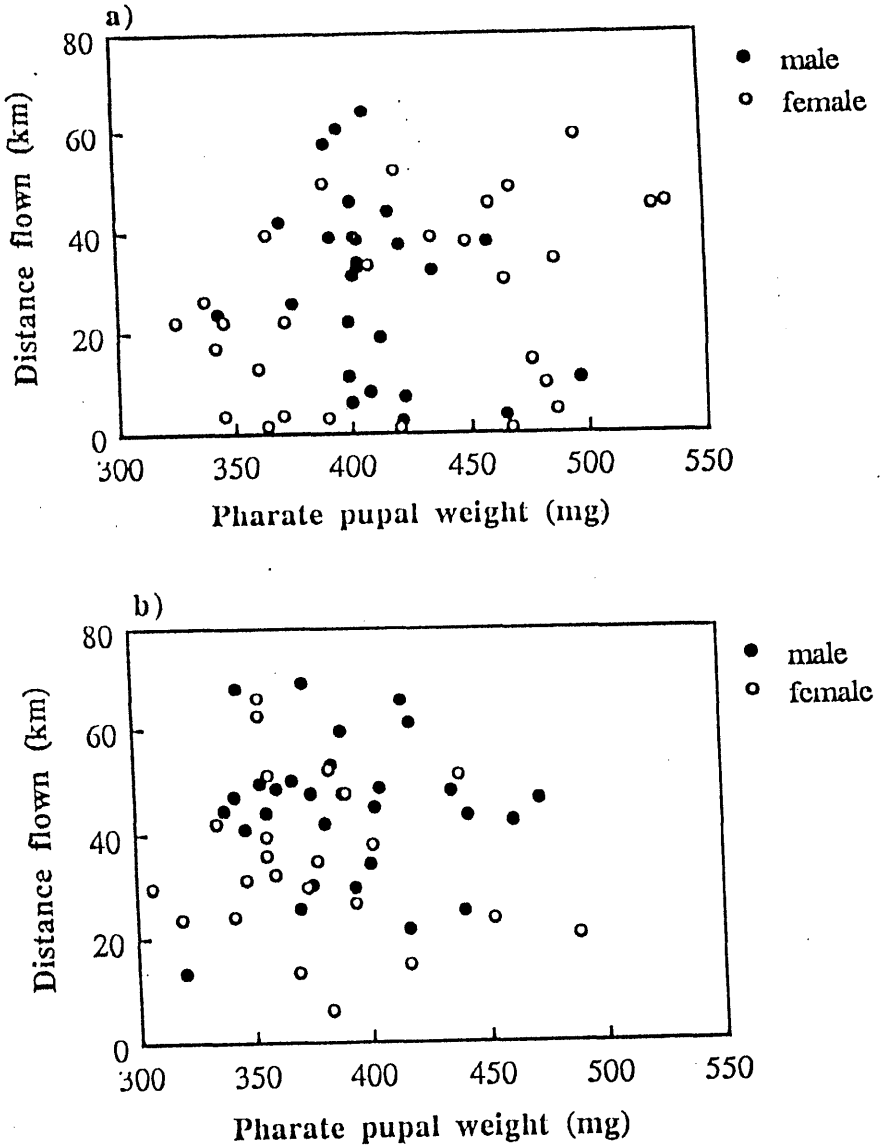

Fig 4.6 Plots of distance flown (km) versus pharate pupal weight (mg) for *H. armigera* males and females on a) night 1, and b) night 4 following emergence.

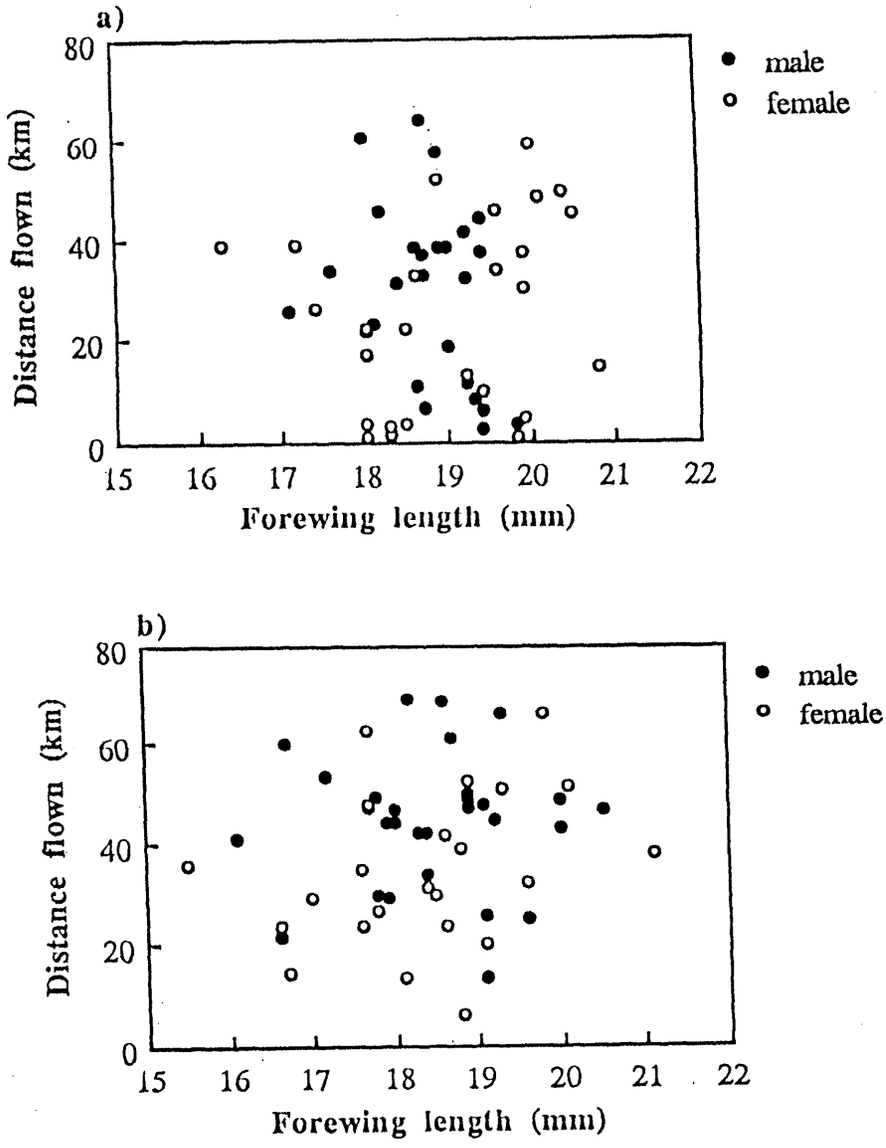

Fig 4.7 Plots of distance flown (km) versus forewing length (mm) for *H. armigera* males and females on a) night 1, and b) night 4 following emergence.

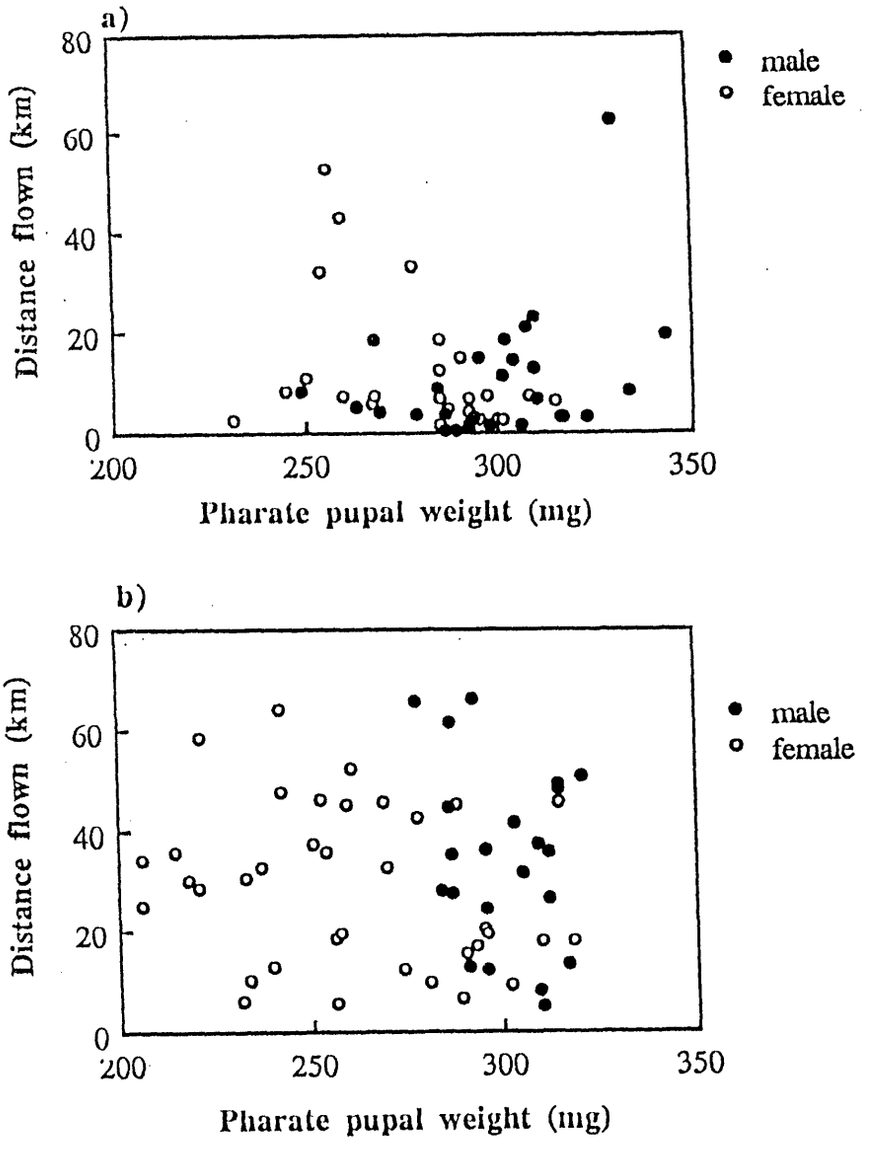

Fig 4.8 Plots of distance flown (km) versus pharate pupal weight (mg) for *H. punctigera* males and females on a) night 1, and b) night 4 following emergence.

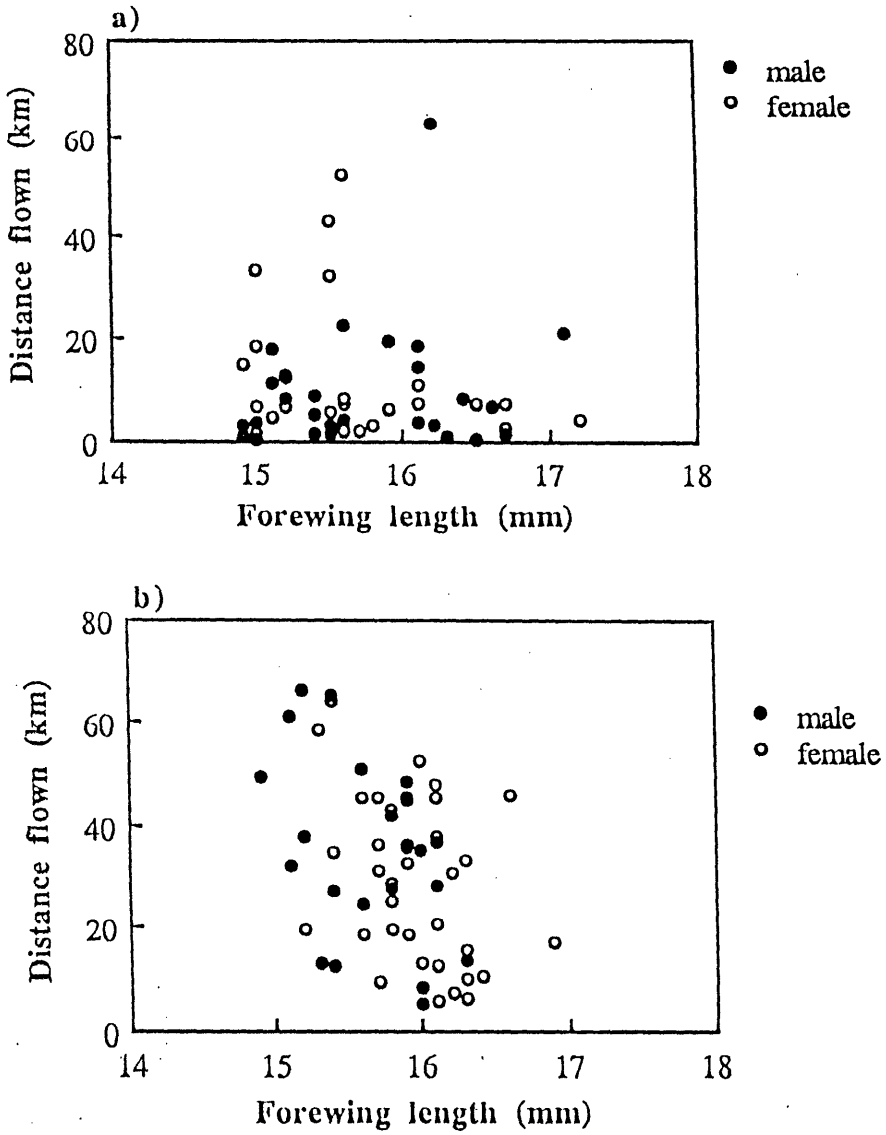

Fig 4.9 Plots of distance flown (km) versus forewing length (mm) for *H. punctigera* males and females on a) night 1, and b) night 4 following emergence.